BIBIANA ALVES DOS SANTOS

"SALT REDUCTION IN DRY FERMENTED SAUSAGES: EFFECTS ON BIOCHEMICAL, CHEMICAL, PHYSICAL AND SENSORY PROPERTIES"

"REDUÇÃO DE SÓDIO EM SALAMES: EFEITO SOBRE AS PROPRIEDADES BIOQUÍMICAS, QUÍMICAS, FÍSICAS E SENSORIAIS"

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

Tese apresentada à Faculdade de Engenharia de Alimentos da Universiade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Tecnologia de Alimentos

Orientadora: Marise Aparecida Rodrigues Pollonio

Co-Orientador: Roger Wagner

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ABSTRACT

The recommendations of the World Health Organization for reducing sodium intake, which has been positively correlated with hypertension and increased risk of cardiovascular disease, have led to the need to reduce salt content, the main source of sodium in the processed products. In fermented meat products such as dry fermented sausages characterized by the high NaCl content, this reduction is a major technological challenge due to its functions including the reduction of water activity, solubilization of myofibrillar proteins, controlling of microbiological growth and sensory properties. Although the use of salt substitutes, such as KCl, $CaCl_2$ and $MgCl_2$ is presented as a strategy for reducing sodium levels, few studies have focused on the biochemical reactions occurring during the process of fermentation, maturation, and shelf life of dry fermented sausages. This study aimed to evaluate the physicochemical, microbiological, biochemical, and sensory stability of dry fermented sausages with reduced NaCl (50%), or NaCl replaced by KCl, $CaCl_2$, and a blend of KCl and $CaCl_2$ during the manufacturing process and throughout 90 days of storage. Overall, the results indicate that the reduction of 50% NaCl and 50% substitution of NaCl by KCl 50% did not change the physicochemical, microbiological, and sensory characteristics during processing. An increase in proteolysis was observed in the samples with 50% NaCl reduction, with a decrease in hardness and firmness during processing and storage. Volatile compounds from lipid oxidation were generated in smaller amounts in this treatment. For the dry fermented sausages produced with 50% NaCl and 50% KCl, both proteolysis and rheological properties were not changed. Volatile compounds from carbohydrates fermentation and amino acid degradation increased during storage. The greatest changes were observed in the formulations containing 50% $CaCl_2$ and blend of 25% KCl and 25% $CaCl_2$.

High water activity, low pH, and a decrease in micrococcaceae counts. During processing and storage, a greater number of volatile compounds from lipid oxidation and low oxidative stability were observed. Furthermore, changes were observed in the electrophoretic profile of sarcoplasmic proteins from the dry fermented sausages containing $CaCl_2$, which also presented an increase in hardness during storage. The sensory quality was adversely affected, once the dry fermented sausages containing $CaCl_2$ were characterized by the attributes rancid aroma and rancid flavor by the descriptive analysis panel. Similar behavior was also obtained by the CATA and Free Listing tests. Thus, it can be concluded that KCl is the most suitable salt to replace NaCl in the formulations, once it did not affect the chemical, microbiological, biochemical, and sensory quality of the fermented meat products.

Keywords: Dry fermented sausage, Sodium chloride reduction, Potassium Chloride, Calcium chloride.

RESUMO GERAL

As recomendações da Organização Mundial da Saúde para redução do consumo de sódio, o qual tem sido positivamente correlacionado com aumento de risco de hipertensão arterial e doenças cardiovasculares, resultam na necessidade de redução do teor de NaCl, principal fonte de sódio nos produtos finais. Produtos cárneos fermentados, como salames, são caracterizados por elevados teores de NaCl, e portanto, tal redução é um grande desafio tecnológico em função de suas funções que incluem redução da atividade de água, solubilização das proteínas miofibrilares, controle do crescimento microbiológico e propriedades sensoriais. O uso de sais substitutos ao NaCl tais como, KCl, $CaCl_2$ e $MgCl_2$ apresenta-se como estratégia para reduzir teores de sódio, no entanto, poucos estudos abordam profundamente as reações bioquímicas ocorridas ao longo do processo de fermentação, maturação e vida útil de salames. O objetivo da pesquisa foi avaliar a estabilidade físico-química, microbiológica, bioquímica e sensorial de salames com redução de NaCl (50%) ou substituídos por KCl, $CaCl_2$ e *blend* de KCl e $CaCl_2$ durante o processamento e vida de prateleira de 90 dias. Os resultados indicaram que a redução de 50% de NaCl e a substituição de 50% de NaCl por 50% de KCl não alteraram as características físico-químicas, microbiológicas e sensoriais durante o processamento dos salames. Foi observado um aumento da proteólise nos salames com 50% de redução de NaCl e uma diminuição da dureza e firmeza dos salames durante o processamento e armazenamento. Os compostos voláteis provenientes da oxidação lipídica foram gerados em menor quantidade neste tratamento. Para os salames produzidos com 50% de NaCl e 50% de KCl, o desenvolvimento da proteólise e as propriedades reológicas não foram alterados. Os compostos voláteis da fermentação de carboidratos e degradação

de aminoácidos aumentaram durante o armazenamento dos salames. A adição de 50% de $CaCl_2$, bem como do *blend* de 25% de KCl e 25% de $CaCl_2$ resultaram em valores mais altos de atividade de água, baixos valores de pH e uma diminuição na contagem de micrococaceas. Durante o processamento e armazenamento dos salames, foi observado um maior número de compostos voláteis provenientes da oxidação lipídica e baixa estabilidade oxidativa. Além disso, os salames adicionados de $CaCl_2$ tiveram o perfil de eletroforese das proteínas sarcoplasmáticas alterado e apresentaram um aumento da dureza durante o armazenamento. A qualidade sensorial foi afetada negativamente e o painel treinado da Análise Descritiva Quantitativa caracterizou os salames adicionados de $CaCl_2$ pelos atributos de aroma e sabor de ranço. De modo semelhante, os testes CATA e Free Listing reportaram este mesmo comportamento por parte dos consumidores. Desta forma, pode-se concluir que o KCl é o sal mais indicado para que a substituição de NaCl não resulte em prejuízos à qualidade físico química, microbiológica, bioquímica e sensorial dos produtos cárneos fermentados.

Palavras-chave: Salame, Redução de Cloreto de Sódio, Cloreto de Potássio e Cloreto de Cálcio.

ÍNDICE

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INTRODUÇÃO GERAL

Introdução Geral

A carne e os produtos cárneos representam uma boa fonte de minerais, tais como ferro, zinco e selênio. No entanto, os produtos cárneos também possuem elevado teor de sódio em suas formulações. A ingestão de sódio em excesso está correlacionada positivamente com o aumento da pressão arterial, doenças cardiovasculares e certos tipos de câncer (HE e MACGREGOR, 2010; DESMOND, 2006).

Por estas razões, nas últimas décadas, órgãos de saúde pública e autoridades regulatórias têm estabelecido programas para promover a redução da ingestão de cloreto de sódio na dieta (FSA, 2006; WHO, 2010; BRASIL, 2013), a fim de diminuir o risco de incidência de doenças crônicas correlacionadas com altos teores de sódio (MATTHEWS e STRONG, 2005). Esse ingrediente é apontado como a mais relevante fonte de sódio na dieta. O impacto dessas recomendações mundias ganha relevância pelo fato de aproximadamente 80% de sódio consumido pelas populações origina-se do consumo de alimentos industrializados (HE e MACGREGOR, 2010). Em países emergentes, como o Brasil, essa contribuição é um pouco menor, já que em refeições domésticas e fora do lar (Serviços de Alimentação), o teor de sal adicionado é bastante significativo (SARNO et al., 2009). De qualquer forma, produtos cárneos são destacados por altos teores de NaCl em suas formulações e, podem ser consumidos também como complementos de refeições principais. Dentre os produtos cárneos processados, os fermentados são destacados por apresentarem valores muito elevados de sódio.

Em alguns países a meta para redução de Na em produtos cárneos fermentados é de 650 mg/100 g de produto (Food Standards Agency (FSA), 2014), enquanto que no Brasil esta categoria de produtos cárneos foi excluída

do último Termo de Compromisso assinado entre Ministério da Saúde e diferentes associações das indústrias brasileiras de alimentação (BRASIL, 2013). As razões para esta medida foram explicadas pela barreira tecnológica, relacionadas à ação antimicrobiana e textura dos produtos.

Certamente, a redução de NaCl em tais produtos representa um grande desafio para a indústria cárnea em função das propriedades multifuncionais desse ingrediente. Conforme demonstrado em alguns estudos (ARMENTEROS et al., 2009; CAMPAGNOL et al., 2012), a redução de NaCl pode alterar a qualidade final de produtos cárneos embutidos fermentados, já que este contribui para a estabilidade microbiólogica, redução da atividade de água, solubilização das proteínas miofibrilares e desenvolvimento de sabor e textura características para esse tipo de produto (LÜCKE, 1998).

Desta forma, estudos conduzidos para promover a substituição ou redução de NaCl em produtos cárneos fermentados vem responder às necessidades da indústria de processamento e atender a demanda de novos consumidores adeptos de alimentos com características mais saudáveis e às solicitações de programas nutricionais governamentais. Algumas abordagens para a redução do teor de sódio em produtos cárneos incluem, por exemplo, a utilização de outros sais em substituição ao cloreto de sódio, tais como KCl, $MgCl_2$ e $CaCl_2$ (FULLADOSA et al., 2009). Dentre estes, o KCl é o mais estudado (GOU et al., 1996; GIMENO, ASTIASARÁN e BELLO, 2001; CAMPAGNOL et al., 2012) por apresentar propriedades físico-químicas similares ao NaCl, porém sua aplicação em elevados níveis de substituição ainda é limitada por seu gosto residual amargo e sabor metálico e adstringente (GOU et al., 1996).

Na literatura, poucas informações são reportadas sobre o efeito da substituição total ou parcial de NaCl por outros sais clorados divalentes, tais

como $MgCl_2$ e $CaCl_2$, no desenvolvimento das reações bioquímicas de produtos cárneos fermentados, especialmente salames. Tais reações, como proteólise, lipólise e oxidação lipídica, que ocorrem em sua maioria durante o processo de fermentação e maturação de produtos cárneos fermentados, contribuem com as características sensoriais de aroma, sabor e textura deste tipo de produto, e portanto, devem ser bem controladas.

Sendo assim, o objetivo do trabalho foi avaliar durante o processamento de salames, a influência da redução ou substituição de 50% de NaCl por 50% de KCl, 50% de $CaCl_2$ e *blend* de 25% KCl e 25% $CaCl_2$ sobre as reações físico-químicas, microbiológicas, bioquímicas e sensoriais ao longo do processamento de salames. E ao longo do armazenamento, as propriedades reológicas, compostos voláteis e estabilidade oxidativa.

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CAPÍTULO 1- Revisão Bibliográfica

IMPORTÂNCIA E DESAFIOS DA REDUÇÃO DE NaCl NO PROCESSAMENTO E QUALIDADE DE PRODUTOS CÁRNEOS FERMENTADOS

1 Cenário atual do consumo de NaCl no mundo

O sódio é um nutriente essencial necessário para o metabolismo celular, onde participa da manutenção do fluido extracelular, e juntamente com o potássio é responsável pela manutenção da homeostase no organismo. Também participa na transmissão de impulsos nervosos e contração muscular (FEINER, 2006). Por outro lado, seu excesso tem efeitos negativos, tais como a hipertensão e consequentemente o aumento da incidência de doenças cardiovasculares (DESMOND, 2006). Outros estudos relatam que indiretamente a alta ingestão de sódio na dieta, pode estar relacionada com doenças como obesidade (HE e MACGREGOR, 2010), cálculos renais, osteoporose e certos tipos de câncer (CAPPUCCIO, KALAITZIDIS e DUNECLIFT, 2000).

A principal fonte de sódio na dieta é proveniente do sal (cloreto de sódio) presente na maioria dos alimentos industrializados (HE e MACGREGOR, 2010). O NaCl é composto por 39,3% de sódio e 60,7% de cloreto, aproximadamente (FEINER, 2006). Pesquisas recentes mostram que grande percentual da população excede o valor diário recomendado pelas autoridades de saúde pública e órgãos regulatórios em diversos países (ALIÑO et al., 2010a; DOYLE e GLASS, 2010; BRASIL 2013), o que justifica ainda mais a implementação da tendência para ingestão de teores mais baixos de sódio.

Em 2008, a Organização Mundial da Saúde (OMS) reduziu o limite de ingestão de sal para 5g/dia/pessoa (2g/dia/pessoa de sódio) (WHO, 2003), anteriormente de 6g/dia/pessoa (FSA, 2006). Esta iniciativa baseou-se nos dados nacionais disponíveis sobre o consumo e níveis de NaCl encontrados nos alimentos, desenvolvimento de ações para sensibilizar os consumidores à respeito dos malefícios do excesso de sódio na dieta, reformulação dos

alimentos industrializados e monitoramento e avaliação destas medidas (ZANARDI et al., 2010). Adicionalmente, em 2010, National Institute for Health and Clinical Excellenc (NICE) recomendou a redução da ingestão de sal para 3g/dia como meta para 2025 para adultos da população do Reino Unido. Na Finlândia, Canadá, Holanda, Austrália, Estados Unidos entre outros países, as medidas para redução de sal nos alimentos acompanham as recomendações da Organização Mundial da Saúde e cada país tem suas próprias medidas e metas determinadas para cumprir estes acordos.

Na América Latina, em 2009, a Organização Pan-Americana de Saúde (OPAS) e o Ministério da Saúde Brasileiro iniciaram as discussões para o monitoramento do consumo de sal e sódio na dieta da população e políticas para sua redução começaram a ser monitoradas (OPAS, 2010). Em 2010, o Brasil através do Ministério da Saúde e Associação Brasileira das Indústrias da Alimentação (ABIA), estabeleceu um compromisso para promover a redução voluntária de sal nos alimentos processados. No entanto, somente em 2011, uma ação conjunta do Ministério da Agricultura, Ministério da Saúde e ABIA através da Anvisa (ANVISA, 2012) definiu metas para a redução de sal nos alimentos. A partir dos dados obtidos pela Pesquisa de Orçamento Familiar 2002-2003 e 2008-2009 (POF) realizada pelo Instituto Brasileiro de Geografia e Estatística (IBGE) as autoridades selecionaram diferentes níveis de redução de sal por categorias de alimentos, que incluem os produtos cárneos (IBGE, 2010).

Os produtos cárneos constituem-se numa categoria de alimentos que apresentam elevado percentual de sódio e representam cerca de 20-30% do sal ingerido na dieta no mundo (WHO, 2010). Em geral, embutidos emulsionados, fermentados e reestruturados apresentam 1,4%; 2,5% e 1% de sal respectivamente (FSA, 2006). Sendo assim, a Food Safety Authority propôs uma

redução média de 40% dos níveis de cloreto de sódio nestes produtos em resposta a procura por alimentos mais saudáveis (FSA, 2006).

Recentemente, novas metas de redução de sal foram estabelecidas pelo Reino Unido juntamente com a Agência de Alimentos (FSA) para o ano de 2017. Neste documento novas categorias de alimentos foram incluídas e a redução entre 40 e 50% de NaCl foram estipuladas para a maioria dos alimentos. Para produtos cárneos em geral, a quantidade média de sal não deve ultrapassar 1,34 g de sal para casa 100g de produto, o que corresponde a 536 mg de sódio a cada 100g de produto (FSA, 2014).

No Brasil, a redução de sal nesta categoria de produtos é bastante sutil e ainda está em fase inicial de implementação. Cabe salientar, que no Termo de Compromisso assinado entre ANVISA, ABIA, Associação Brasileira das Indústrias de Queijos (ABIQ), Associação Brasileira da Indústria Produtora e Exportadora de Carne Suína (ABIPECS), Sindicato da Indústria de Carnes e Derivados no Estado de São Paulo (SINDICARNES) e União Brasileira de Avicultuta (UBABEF), em 2013, a redução de NaCl na categoria de produtos cárneos fermentados foi excluída do acordo. Segundo o documento, produtos cárneos fermentados possuem barreiras tecnológicas para a redução de NaCl, como ação antimicrobiana e textura dos produtos. Por outro lado, entidades e órgãos, de saúde pública de outros países incluem a redução de NaCl nos embutidos fermentados para no máximo 1,63% de NaCl como meta para 2017, o que representa uma redução média de 35% de NaCl se considerarmos a quantidade de 2,5% de NaCl de uma formulação tradicional (FSA, 2014).

No cenário internacional, principalmente em países europeus, o consumo de embutidos fermentados é bastante elevado e as pesquisas (ZARNARDI et al., 2004; GUÀRDIA et al., 2008; RIPOLLÉS et al., 2011; CORRAL et al., 2013)

colaboram para que estes produtos possam ter seu teor de NaCl reduzido. No Brasil, os salames são os mais consumidos entre os produtos cárneos fermentados, e sendo assim representam uma oportunidade para que novas pesquisas sejam desenvolvidas a fim de reduzir o teor de NaCl nestes produtos.

Diversas pesquisas (GOU et al., 1996; GIMENO, ASTIASARÁN e BELLO, 2001; GUÀRDIA et al., 2006) apontam a dificuldade de reduzir o teor de sal nos embutidos fermentados, uma vez que o cloreto de sódio além de proporcionar o gosto salgado característico, desempenha importantes funções tecnológicas e influencia a estabilidade microbiológica (WIRTH, 1991; RUUSUNEN e PUOLANNE, 2005). Desta forma, estudar a redução e/ou substituição de NaCl em produtos cárneos fermentados torna-se importante para que futuramente possa fazer parte do plano de metas de redução de NaCl no Brasil.

2 Funções do cloreto de sódio e processamento de produtos cárneos fermentados

O cloreto de sódio (NaCl) é um ingrediente essencial no processamento de produtos cárneos fermentados, contribuindo de forma efetiva sobre as características tecnológicas, sensoriais e de estabilidade dos produtos (GOU et al., 1996; DESMOND, 2006). Uma das mais importantes funções do sal é a extração das proteínas miofibrilares as quais são solúveis somente em alta força iônica. Essa classe de proteínas constituída predominantemente pelo complexo acto-miosina é responsável pelo desenvolvimento de propriedades funcionais que caracterizam os produtos cárneos processados. No caso de produtos fermentados, contribuem na formação de emulsão e gelificação, processos que

terão papel fundamental nas etapas de acidificação e posterior secagem dos produtos cárneos fermentados (RUIZ, 2007).

Outra função de grande relevância do NaCl está associada à estabilidade microbiológica. Este atua como um agente bacteriostático e inibe o crescimento microbiológico. O principal mecanismo pelo qual o cloreto de sódio atua como conservante está relacionada à sua capacidade de reduzir a atividade de água e, consequentemente, promover a desidratação osmótica do produto cárneo (BEUCHAT citado por SAMAPUNDO et al., 2010). O efeito conservante do cloreto de sódio está correlacionado com a quantidade adicionada, e em produtos cárneos fermentados, pode chegar a 6% no produto final (LÜCKE, 1998). Esta concentração é suficiente para controlar o crescimento microbiológico, ao favorecer o crescimento de microrganismos gram-positivos (*Lactobacillus*, *Micrococcus* e *Pediococcus*) inibir gram-negativos (*Pseudomonas spp*., *Salmonella spp*. e *Escherichia coli*) e ainda pode ser tóxico para algumas bactérias por criar um desequilíbrio eletrolítico dentro da célula bacteriana (LÜCKE, 1998).

Primeiramente, o NaCl irá atuar sobre a microbiota contaminante presente naturalmente na matéria-prima cárnea. Neste momento sua ação será sobre os microrganismos psicotrópicos e bactérias gram-negativas (especialmente *Pseudomonas)*. Esta microbiota aos poucos começa a diminuir, o que permite um rápido crescimento de bactérias ácido láticas gram positivas. Este fato demonstra a ação essencial do NaCl sobre a fermentação, visto que a queda do pH destes produtos é favorecida nestas condições (SEBRANEK, 2012). Com a queda do pH, o processo de extração das proteínas miofibrilares, a redução da atividade de água, e a desidratação dos produtos é favorecida (LÜCKE, 2000).

A textura típica de produtos cárneos fermentados, uma das propriedades físicas e sensorial mais importantes que define a qualidade do produto final, resulta da redução do pH e atividade de água que ocorrem nas etapas de fermentação e maturação ao longo do processo (TOLDRÁ, 2006). Dessa forma, todas as funções do sal citadas acima irão contribuir para o desenvolvimento final da textura dos produtos. Resumidamente, a adição de sal durante a cominuição das carnes e gordura auxilia na solubilização das proteínas musculares que coagulam e formam um gel em torno das partículas de carne e gordura. Este processo é auxiliado pelo desenvolvimento de bactérias ácido láticas que irão reduzir o pH para próximo do ponto isoelétrico das proteínas que por sua vez irá iniciar o processo de liberação de água. Neste período, o gel formado é estabilizado e uma matriz de carne e gordura determina a textura típica de embutidos fermentados (TALON, 2004).

Diante da importância do NaCl no processo tecnológico, estabilidade microbiológica e desenvolvimento de propriedades sensoriais características dos produtos cárneos fermentados, o desafio na redução de sal nestes produtos requer o estudo de diferentes estratégias para que o produto final tenha caraterísticas similares aos produtos tradicionais. Vários estudos, ao substituir diferentes níveis de NaCl, reportaram uma alteração no perfil de textura dos produtos cárneos fermentados (TERRELL et al., 1981; GOU et al., 1996; GELABERT et al., 2003; RUUSUNEN e PUOLANNE, 2005; GUÀRDIA et al., 2008; ALIÑO et al., 2010b, SANTOS et al., 2014).

De modo semelhante, a alteração da cor dos produtos cárneos com redução de NaCl foi reportada em vários trabalhos (GOU et al., 1996; GIMENO, ASTIASARÁN e BELLO, 1999; ZANARDI et al., 2010) e tal mudança pode

ocorrer devido a diferenças de pH resultantes da substituição de cloreto de sódio por outros sais (HAMM citado por GOU et al., 1996).

Uma das contribuições mais relevantes do cloreto de sódio é o desenvolvimento de gosto salgado característico de produtos cárneos (RUUSUNEN e PUOLANNE, 2005; MILLER e BARTHOSHUK, 1991). A percepção do gosto salgado tem início quando os íons sódio ativam os receptores de sabor (ENaCs) e um estimulo nervoso é enviado para regiões do cérebro responsável pelo processamento gustativo (KEAST e ROPER, 2007). A intensidade do estímulo nervoso dependerá da interação do sentido do gosto e da concentração de sódio até um limite máximo limiar terminal de reconhecimento (KEAST e ROPER, 2007). Este mecanismo pode ajudar a explicar a baixa percepção do gosto salgado em alimentos com reduzido teor de NaCl. A redução de sal além de reduzir o gosto salgado, pode resultar em uma menor percepção de sabor, bem como ao aumentar o gosto amargo e resultar em diferentes sabores devido as interações com outros sais substitutos ao NaCl (BRESLIN e BEAUCHAMP, 1997; KEAST, DALTON e BRESLIN, 2004).

A percepção do gosto salgado e sua intensidade dependem da quantidade de sal, tipo e composição do produto cárneo. As características de cada produto, como teores de gordura, formação de complexos entre os ânions e proteínas miofibrilares, presença de diferentes aminoácidos, substâncias procedentes da proteólise podem alterar o gosto salgado dos produtos cárneos fermentados (ARISTOY e TOLDRÁ, 1995). Alguns estudos comprovam que o teor de gordura do produto cárneo altera a percepção de salinidade proporcionada pelo cloreto de sódio (MATULIS et al., 1995; RUUSUNEN, TIRKKONEN e PUOLANNE, 2001; RUUSUNEN e PUOLANNE, 2005) e quanto

maior o teor de gordura, mais evidente se torna o gosto salgado (DESMOND, 2006).

Os defeitos na qualidade sensorial de embutidos fermentados com teor reduzido de NaCl também podem ser explicados por variações no tempo de maturação. Nessa fase, ocorre intensa atividade de reações químicas e bioquímicas, nas quais enzimas lipolíticas e proteolíticas alteram a textura e são responsáveis pela formação de compostos voláteis (peptídeos e aminoácidos livres). Esses metabólitos podem conferir sabor e aroma desagradável, ao reforçar o sabor metálico e gosto amargo de alguns sais de cloreto (TOLDRÁ, 1998; TOLDRÁ, ARISTOY e FLORES, 2000). De um modo geral, pesquisas indicam que a substituição entre 25-50% de cloreto de sódio em produtos cárneos é aceitável em relação à percepção do sabor (PRICE, 1997; GUÀRDIA et al., 2006), mas vários fatores parecem influenciar essa faixa, tais como seleção da matéria prima, teor de gordura e condições de processamento.

A modificação de qualquer ingrediente ou processo tecnológico de fabricação pode alterar a solubilidade das proteínas, e consequentemente prejudicar o desenvolvimento da textura final do produto. Além disso, uma perda da solubilidade das proteínas miofibrilares pode interferir nos processos proteolíticos, diminuir a capacidade de retenção de água e afetar o processo de desidratação do produto cárneo fermentado. Nestes produtos, a redução de cloreto de sódio pode requerer uma adaptação do processo de cura como adequação dos parâmetros de umidade relativa e temperatura da câmara de maturação (WIRTH, 1991).

3 Efeitos do NaCl sobre a proteólise

O efeito do sal sobre o sistema proteolítico muscular vem sendo abordado amplamente nas últimas décadas. A influência do conteúdo de sal sobre a atividade de algumas enzimas proteolíticas, como as catepsinas e algumas proteases, tem sido demonstrada (RICO, TOLDRÁ e FLORES, 1991; TOLDRÁ, RICO e FLORES, 1993; FLORES, ARISTOY e TOLDRÁ, 1997; SENTANDREU, TOLDRÁ, 2001a; TOLDRÁ, 2002).

As reações de proteólise (Figura 1) constituem-se num importante mecanismo bioquímico durante o processamento de produtos cárneos, especialmente nos processos que envolvem fermentação e maturação, onde o controle do processo é fundamental para obter um resultado benéfico na qualidade final do produto (TOLDRÁ, RICO e FLORES, 1993). Os principais produtos da proteólise são pequenos peptídeos e aminoácidos livres que influenciam o sabor e textura (TOLDRÁ, 1998). Outros compostos gerados a partir de aminoácidos através de outras reações enzimáticas e não-enzimática também podem contribuir indiretamente para o desenvolvimento de aroma (TOLDRÁ, RICO e FLORES, 1993).

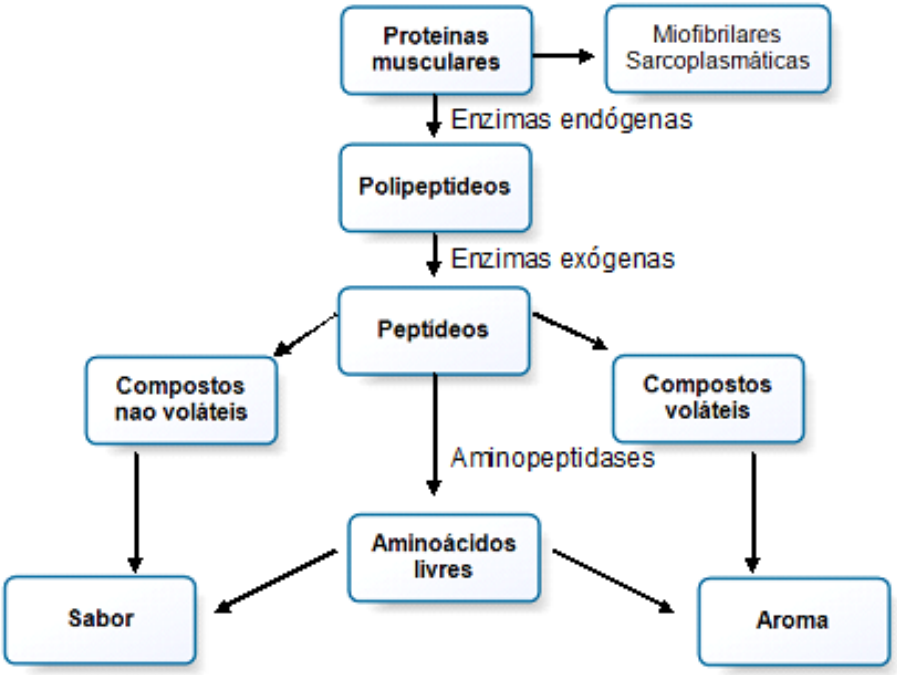

Figura 1- Cadeia proteolítica de produtos cárneos fermentados.

Fonte: Adaptado de Toldrá, 1998.

As enzimas envolvidas na proteólise são as endoproteases, principalmente calpaínas e catepsinas, responsáveis pela degradação de proteínas (YATES et al., 1983; KOOHMARAIE, 1994) e as exopeptidases, principalmente dipeptidilpeptidases e aminopeptidases (TOLDRÁ, 2006). Todas elas têm papel importante na cadeia proteolítica, especialmente no processo de maturação, já que originam uma série de produtos finais (peptídeos e aminoácidos livres) envolvidos no desenvolvimento de aroma e sabor dos produtos cárneos fermentados (TOLDRÁ, 2006).

As endopeptidases são enzimas proteolíticas tissulares responsáveis principalmente pela degradação das proteínas miofibrilares e sarcoplasmáticas (TOLDRÁ, 2006). O resultado de sua atividade é a geração de fragmentos protéicos e peptídeos procedentes em sua maioria da quebra das proteínas miofibrilares, actina e miosina, que irão servir de substrato para a ação das

exopeptidases (TOLDRÁ, 1998; TOLDRÁ, 2006). Dentro deste grupo, estão as calpaínas, catepsinas e proteosomas. As calpaínas e catepsinas são bastante estáveis durante o processo de fabricação dos embutidos fermentados. No entanto, agentes de cura como o cloreto de sódio inibem a atividade destas enzimas e, portanto, auxiliam no controle da degradação proteica (TOLDRÁ, 2006).

As exopeptidases são um grupo de enzimas proteolíticas caracterizadas por hidrolisar as cadeias peptídicas através de seus grupamentos terminais, ao proporcionar a liberação de pequenos peptídeos e aminoácidos livres (TOLDRÁ, 2006). Este grupo é representado por tripeptidilpeptidases, dipeptidilpeptidases e carboxipeptidases, entre outros (TOLDRÁ, 1998). Os aminoácidos livres têm grande importância no desenvolvimento do gosto característico dos embutidos fermentados. Também participam das reações de degradação, que geram compostos voláteis que contribuem para o sabor e aroma (TOLDRÁ, ARISTOY e FLORES, 2000).

A formação de aminoácidos e peptídeos também pode ser proveniente da ação dos microrganismos adicionados nos embutidos fermentados. O sistema proteolítico das bactérias ácido láticas está associado à conversão de proteínas em oligopeptídeos (KUNJI et al., 1996; SIEZEN, 1999). No entanto, a atividade das culturas *Starters* é considerada baixa quando comparada as enzimas tissulares (KUNJI et al., 1996; SIEZEN, 1999).

A contribuição dos aminoácidos livres sobre o aroma e sabor dos embutidos fermentados depende da quantidade e equilíbrio entre os aminoácidos presentes. Uma das principais contribuições dos aminoácidos livres é o sabor de carne curada. Os ácidos glutâmico e aspártico são gerados em grandes quantidades em embutidos fermentados e podem conferir um gosto

ácido. A fenilalanina, triptofano e tirosina também se apresentam em elevada concentração e quando sua formação não é bem controlada podem depreciar a qualidade sensorial dos produtos ao desenvolver gosto amargo. Por outro lado, os aminoácidos alanina, serina, prolina, glicina, hidroxiprolina tem a capacidade de modular o sabor dos embutidos fermentados através do gosto doce (ARISTOY e TOLDRÁ, 1995).

As reações de proteólise dependem de muitos fatores que afetam o perfil das enzimas musculares, como as características próprias da matéria-prima (TOLDRÁ, 1998), a idade dos animais (TOLDRÁ, 1998), tipo de músculo utilizado no processamento (ARISTOY e TOLDRÁ 1995), formulação do produto e tipo de cultura *Starter* (HUGHES, COFRADES e TROY, 1997). Outros fatores importantes estão relacionados à tecnologia de processamento, como a temperatura e o tempo de maturação, que têm um efeito direto sobre a atividade das enzimas e do sal presente no produto, exercendo um grande efeito sobre as proteases musculares (RICO, TOLDRÁ e FLORES, 1991; ROSELL e TOLDRÁ, 1996; SENTANDREU e TOLDRÁ, 2001b).

A maioria das enzimas envolvidas na proteólise muscular de produtos cárneos fermentados é fortemente afetada pela diminuição da atividade de água, que tem impacto principalmente na ação das catepsinas e aminopeptidases (TOLDRÁ, 2006). Além disso, o teor de sal, o qual aumenta ao longo do período de maturação resultante da redução da Aw, inibe parcialmente a atividade das enzimas microbianas e atividade das aminopeptidases (SENTANDREU e TOLDRÁ, 2001b; TOLDRÁ, MIRALLES e FLORES, 1992; TOLDRÁ, RICO e FLORES, 1993). Poucos estudos abordam a redução de cloreto de sódio e suas reações de proteólise ao longo do processo de fermentação e maturação. Toldrá (1998) e Toldrá, Miralles e Flores (1992) explicam que as catepsinas são inibidas

pelo aumento da concentração de sal dos produtos cárneos fermentados. Poucos estudos abordam o efeito que a redução ou substituição de NaCl promove sobre a atividade destas enzimas. García-Garrido et al. (2000) relataram que presuntos crus com redução de sal tiveram uma alta atividade da enzima catepsina B, prejudicando a textura final dos produtos. De forma similar, Roseiro et al. (2008) ao estudarem as reduções de 3 e 6% de cloreto de sódio em embutidos fermentados, observaram maior degradação de miosina nos produtos com menor teor de sal. De um modo geral, os estudos indicam que a intensidade da proteólise é alterada quando o teor de NaCl é reduzido ou substituído por sais de cloreto (RIPOLLÉS et al., 2011; DOS SANTOS et al., 2015).

Segundo Toldrá (2006), um excesso de proteólise pode afetar negativamente as características sensoriais dos produtos cárneos fermentados. O acúmulo de certos compostos nitrogenados de baixo peso molecular (peptídeos e aminoácidos) pode desenvolver gosto amargo e sabor metálico. A presença de cristais brancos de tirosina deprecia a aparência dos produtos e a textura pode ficar excessivamente macia devido à degradação excessiva as proteínas miofibrilares.

4 Efeitos do NaCl sobre as reações lipolíticas

As reações de lipólise e oxidação lipídica têm sido amplamente estudadas em produtos cárneos fermentados (MOLTILVA et al., 1994; ZANARDI et al., 2004; RIPOLLÉS et al., 2011) com a finalidade de entender o efeito destas reações sobre a qualidade e segurança dos produtos. Em geral, estas reações estão implicadas no processo de deterioração de muitos alimentos. No entanto,

em produtos cárneos fermentados estas reações, quando controladas, desempenham papel fundamental na qualidade sensorial (ARMENTEROS, 2010), principalmente no desenvolvimento de aroma e sabor característicos (ZANARDI et al., 2010).

A composição lipídica dos produtos cárneos é de principalmente ácidos graxos saturados (SFAs) e monoinsaturados (MUFAs). Já, o teor de ácidos graxos insaturados (PUFAs) dependerá do tipo e quantidade de matéria-prima cárnea utilizada no processamento. Para carne suína, principal matéria-prima de embutidos fermentados, este nível fica entre 10-15% do total de ácidos graxos (VALSTA, TAPANAINEN e MÄNNISTÖ, 2005).

As mudanças que ocorrem com os lipídios interferem principalmente na formação de sabor (CHIZZOLIN, NOVELLI e ZANARDI, 1998). As reações de lipólise e oxidação lipídica são interpretadas como diferentes fenômenos, onde a ação dos microrganismos tem maior impacto sobre a oxidação dos produtos da lipólise (VERPLAETSE, 1994). Outros fatores como pH, atividade de água, presença de metais, nitrito, sal e condições de estocagem podem influenciar diretamente a qualidade organoléptica dos embutidos fermentados (DEMEYER et al., 1974).

As reações de lipólise consistem na quebra dos triacilglicerois por lipases e fosfolipídios por fosfolipases que resultarão em ácidos graxos livres (TOLDRÁ, 2006). As enzimas endógenas, como as lipases mitocondriais e lisossômicas denominadas lipase ácida, fosfolipase A1 e A2, lipase neutra, esterase neutra e esterase ácida que estão presentes na matéria-prima cárnea e gordura animal, são as principais responsáveis pelas reações de lipólise dos produtos embutidos fermentados (JOHANSSON et al., 1994) por promoverem a liberação de mais de 60% dos ácidos graxos livres do produto (MORRISSEY et al., 1998). Estas

enzimas apresentam uma boa estabilidade durante o processo de fabricação, mas sua atividade depende do valor de pH, concentração de sal e atividade de água do produto (JAYATHILAKAN et al., 2007).

Lipase ácida é uma das enzimas inibidas pela diminuição dos teores de cloreto de sódio em embutidos fermentados. Sua principal atividade consiste na hidrólise de mono, di e triacilglicerois na faixa de pH entre 4,5 e 5,5 e no período de secagem, sua ação é favorecida pelo aumento da concentração de cloreto de sódio e diminuição da atividade de água do produto. A lipase neutra, por sua vez, possui ação limitada nos produtos cárneos fermentados, pois atua em faixa de pH entre 7,0 e 7,5 (JOHANSSON et al., 1994).

As enzimas fosfolipases atuam principalmente na fase de maturação dos salames (DEMEYER, HOOZE e MESDOM, 2006), onde a degradação dos fosfolipídios aumenta a geração de ácidos graxos livres como oleico, linoleico, esteárico e palmítico (VENTANAS et al., 2007). Em geral, processos de maturação longos com condições brandas de secagem permitem maior atividade lipolítica e, assim, maior formação de ácidos graxos livres (CHIZZOLINI, NOVELLI e ZANARDI, 1998).

Geralmente, a taxa de geração de ácidos graxos livres, especialmente os ácidos linoleico, esteárico e palmítico aumentaram durante o processo de fabricação de embutidos fermentados (TOLDRÁ, 2002). Algumas pesquisas reportaram que os níveis de ácidos graxos livres aumentaram de 0,7-1,5% para 2,2-4,5% ao final do processo de fabricação (ZANARDI et al., 2004). Por outro lado, Stahnke (1995) e Quintanilla et al. (1996) avaliaram o efeito da substituição de NaCl por KCl sobre a lipólise de embutidos fermentados e observaram uma possível influência inibitória sobre as reações, ao verificarem a redução dos níveis de ácidos graxos saturados livres. Segundo a literatura, o NaCl atua como

um potente pró-oxidante em carnes e produtos cárneos, acelerando as reações de oxidação lipídica embora seu mecanismo de ação não seja completamente esclarecido (KANNER et al., 1991). Existem várias tentativas para explicar a ação pró-oxidante do NaCl e entre elas podemos citar o aumento da atividade das oxidases, modificação das proteínas heme ou ação dos íons cloreto sobre os lipídeos (KANNER et al., 1991; LOVE e PEARSON, 1974). Também a interferência da adição de nitrato e/ou nitrito de sódio sobre a lipólise ainda não é bem esclarecida (STAHNKE, 1995; QUINTANILLA et al., 1996; ZANARDI et al., 2004).

A contribuição da microbiota bacteriana no desenvolvimento da lipólise é considerada limitada, mas pode ser relevante em relação ao tipo e processamento de cada produto (JOHANSSON et al., 1994). Dentre a microbiota presente nos embutidos fermentados, a família das *Micrococcaceae* é considerada uma das mais importantes para a lipólise, pois são capazes de hidrolisar os triacilglicerois (SORENSEN, 1997; SUNESEN e STAHNKE, 2003). Já o grupo predominante de bactérias ácido láticas tem uma atividade lipolítica inferior (SUNESEN e STAHNKE, 2003; SORENSEN, 1997). Deve-se ainda considerar que a produção e atividade das lipases depende da matéria-prima, pH, teor de cloreto de sódio e condições de fermentação e maturação do produto (ZANARDI, 2010).

A oxidação lipídica é um processo complexo e em produtos cárneos fermentados ocorre principalmente pelo mecanismo de autoxidação (FRANKEL, 2005). Neste processo, a oxidação dos ácidos graxos ocorre pelo mecanismo de reação em cadeia de radicais livres (TOLDRÁ, 2002) e os ácidos graxos poli-insaturados são mais susceptíveis quando comparados aos ácidos graxos monoinsaturados. As reações de oxidação lipídica contribuem na formação do

sabor típico de produtos cárneos fermentados e fase inicial desse conjunto de reações está associada ao desenvolvimento de aroma. No entanto, seu excesso pode conduzir ao desenvolvimento de *off-flavors*.

A formação de radicais livres, etapa de iniciação para a ocorrência das reações em cadeia, é catalisada por enzimas oxidativas que estão presentes no músculo, como as peroxidases e ciclooxigenases. Adicionalmente, a presença de luz, aquecimento, umidade e cátions também catalisam a reação. A seguir, a formação de radicais peróxido ocorre na chamada etapa de propagação onde as reações entre os radicais livres e o oxigênio ocorrerão. Os hidroperóxidos, produtos primários da oxidação lipídica, não produzem odor e sabor, entretanto, são bastante reativos e formam compostos secundários que contribuem com o sabor dos embutidos fermentados (NIELSEN et al., 1997). Na etapa final do processo de oxidação, os radicais livres reagem entre si e os principais produtos da oxidação lipídica são aldeídos, hidrocarbonetos alifáticos, alcoóis e cetonas. Todo este processo precisa ser bem controlado para que a oxidação lipídica não ocorra em excesso e leve a perda da segurança e valor nutricional do produto, além de afetar a cor, textura e outras propriedades funcionais (SHAHIDI, 2002).

Em produtos cárneos fermentados, o tipo de cultura *Starter* e o tempo de maturação, bem como o tipo de especiarias utilizadas na formulação, podem alterar a estabilidade oxidativa dos produtos (EDWARDS et al., 1999). Vários estudos reportam a importância da oxidação lipídica sobre o desenvolvimento de características sensoriais, principalmente no desenvolvimento de aroma de embutidos fermentados (JOHANSSON et al., 1994; STAHNKE, 1994; MEYNIER et al., 1999).

5 Efeito do NaCl sobre a geração de compostos voláteis

A geração de compostos voláteis em produtos cárneos fermentados ocorre a partir de carboidratos, proteínas e lipídeos através de reações de degradação de carboidratos, aminoácidos (descarboxilação, desaminação e transaminação), proteólise, lipólise e oxidação lipídica (TOLDRÁ, 2008). Além disso, a adição de especiarias como pimenta, noz- moscada, alho, entre outros conferem sabor e exerce grande impacto no aroma dos embutidos fermentados (ORDOÑEZ et al.,1999).

Todas estas reações estão relacionadas com as mudanças de pH, atividade de água, umidade e textura do produto. Os parâmetros de processamento como umidade, temperatura e tempo de secagem, além do tipo de matéria-prima cárnea e cultura *Starter* utilizada no processamento interferem na composição de compostos voláteis dos embutidos fermentados (DEMEYER, 2004). Adicionalmente, o calibre dos embutidos pode afetar a qualidade e tipo de compostos voláteis gerados durante a fabricação dos produtos (DEMEYER, 2004). Desta forma, o aroma característico de produtos cárneos fermentados poderá ser positivo ou negativo dependendo do tipo, quantidade e equilíbrio entre os compostos voláteis formados (STAHNKE, 1999).

Os carboidratos servem de substrato para o crescimento de micro-organismos adicionados na forma de cultura *Starter* em embutidos fermentados. A sua fermentação produz ácido lático que reduz o pH dos produtos durante a fermentação. Os produtos finais deste metabolismo microbiano são compostos voláteis como acetato, etanol, ácido acético, butanediol, entre outros. Estes compostos irão interferir na qualidade do *flavor* dos produtos cárneos fermentados (TOLDRÁ, 2008). O teor de sal bem como condições e tempo de processamento interferem na geração destes compostos. Em um estudo

desenvolvido por Wu et al. (2015) a elevada substituição de NaCl por KCl em bacon, um produto cárneo curado, acarretou em um aumento da quantidade de 2,3-butanodiona, um composto proveniente da fermentação de carboidratos por micro-organismos.

Os compostos derivados da proteólise geram peptídeos de baixo peso molecular e aminoácidos livres através de inúmeras reações, os quais são responsáveis pela fração não volátil e contribuem com o desenvolvimento do gosto doce, salgado, ácido, amargo e também umami (ORDOÑEZ et al., 1999). A contribuição na formação de aroma é devido à geração de aldeídos de cadeia ramificada e seus produtos secundários como ácidos, álcoois e ésteres (TOLDRÁ, 2006). Os peptídeos, di e tri peptídeos especialmente, resultam da ação de enzimas microbianas e musculares e estas são ativadas ou inibidas dependendo do pH e nível de sal (SENTANDREU e TOLDRÁ, 2001b). Segundo Martín et al. (1998), a adição de NaCl pode controlar a proteólise em produtos cárneos fermentados ao controlar a atividade das enzimas proteolíticas. Estudos recentes demonstram que a redução de NaCl em salames modifica a geração de compostos provenientes da degradação de aminoácidos ao reduzir a quantidade destes compostos (CORRAL et al., 2013). Os compostos mais abundantes reportados em salames com alto teor de sal (2,7% de NaCl) foram o 3-metiltiofeno e dimetil dissulfeto. De forma similar, Wu et al. (2015) observaram um decréscimo do composto 3-metilbutanal em bacon com 30% de NaCl e 70% de KCl, que demonstra o efeito da redução de sal sobre a intensidade da proteólise em produtos cárneos curados.

A fração volátil mais abundante nos embutidos fermentados é proveniente as reações de oxidação lipídica. Um dos compostos mais importantes é o hexanal que quando produzido em excesso pode afetar negativamente o aroma

e sabor dos embutidos fermentados, ao desenvolver um aroma de ranço. Geralmente, a oxidação lipídica origina aldeídos e compostos tais como alcanos, cetonas e álcoois. Purriños et al. (2012) estudaram a geração de compostos voláteis em lombo curado tipo “Lacón” com diferentes tempos de salga. Os lombos com maior tempo de salga (4 dias), e consequentemente com maior teor de sal, apresentaram um aumento da quantidade de compostos voláteis proveniente da oxidação lipídica quando comparados aos tratamentos com menor tempo de salga (2 e 3 dias). Os principais compostos formados foram hexanal, pentanal, heptanal e nonanal. Estes resultados estão de acordo com estudos anteriores que demonstraram o efeito pró-oxidante do NaCl em produtos cárneos (KANNER, HAREL e JOFFE, 1991; PÉREZ-JUAN, FLORES e TOLDRÁ, 2006).

Corral et al. (2013) estudaram o efeito do NaCl sobre a geração de compostos voláteis e um maior número de compostos provenientes da oxidação lipídica foram observados nos salames contendo 2,26% de NaCl e 0,43% de KCl em comparação com os salames contendo 2,7% e 2,26% de NaCl. Os compostos mais abundantes foram hexanal, butanal, 2-etilfurano, 1-pentanol e 2-octenal.

6 Estratégias para reduzir o teor de NaCl em produtos cárneos fermentados

Na literatura, estudos indicam que a simples redução de NaCl em produtos cárneos fermentados, especialmente em salames, não é viável em níveis significativos. Aaslyng, Vestergaard e Koch (2014) não conseguiram reduzir em 25% o teor de NaCl em salames sem alterar a qualidade microbiológica e sensorial dos produtos. Sendo assim, o uso de estratégias como

substituição de NaCl por outros sais de cloreto têm sido estudadas a fim de reduzir o teor de NaCl nesta categoria de produtos cárneos.

6.1 Substituição Parcial de NaCl por Cloreto de Potássio

Um dos sais de cloreto mais utilizados como substituto ao NaCl é o cloreto de potássio (KCl). Recentemente, a Organização Mundial da Saúde (WHO, 2012) inseriu no novo guia de recomendações a ingestão mínima de 3510 mg de potássio por dia. Segundo a WHO, a ingestão de potássio pode diminuir o risco do desenvolvimento de doenças não transmissíveis, como hipertensão e doenças cardiovasculares e desta forma minimizar as consequências negativas do alto consumo de sódio na dieta (WHO, 2012).

O uso de KCl como sal substituto ao NaCl em produtos cárneos tem sido amplamente estudado nas últimas décadas (GOU et al., 1996; GIMENO et al., 2001; GUÀRDIA et al., 2006; CAMPAGNOL et al., 2011a; DOS SANTOS et al., 2015). Este sal é importante porque desempenha função tecnológica e de estabilidade microbiológica semelhante ao NaCl (ASKAR, EL-SAMAHY e TAWFIK, 1994). Um dos primeiros estudos que abordaram a substituição de NaCl por KCl em produtos cárneos fermentados foi desenvolvido por Gou et al. (1996). Os valores de pH não diferiram durante o processo de fabricação dos salames, porém um aumento da dureza foi percebido quando a substituição de NaCl por KCl foi de 40, 50 e 60%. Ibañez et al. (1996) não encontraram diferença entre salames produzidos com 3% de NaCl e a mistura de 1,5% de NaCl e 1% de KCl.

Um estudo realizado por Comaposada, Arnau e Gou (2007) demonstraram que a substituição molar de 35% de NaCl por KCl não influenciou

o processo de secagem de presunto cru. Aliño et al. (2009) substituíram em 35, 50 e 70% NaCl por KCl em lombo suíno curado e apenas na formulação com 70% de KCl foi observada mudança nas propriedades físico-químicas e textura dos produtos. Campagnol et al. (2011b) observaram que a substituição de 25 e 50% de NaCl por KCl não interferiu nos valores de atividade de água, pH, perda de peso e contagem de bactérias láticas e micrococaeas durante a fabricação de salames. De modo semelhante, Santos et al. (2014) observaram que a substituição de 50 e 75% de NaCl por KCl não interferiu no processo de fermentação e maturação de produto embutido fermentado cozido (tipo pepperoni). Por outro lado, a substituição de 75% de NaCl por KCl modificou a coloração e os parâmetros de textura dos produtos reformulados. A qualidade sensorial também foi depreciada por esta substituição.

Outra abordagem bastante importante para a qualidade de produtos cárneos fermentados é o estudo das reações bioquímicas que ocorrem durante a fabricação dos produtos em formulações contendo sais substitutos. O efeito da substituição de NaCl por KCl sobre as reações proteolíticas foi estudada por Armenteros et al. (2009) em presunto cru. Foi observada uma intensa degradação das proteínas miofibrilares, actina e miosina, na fase de maturação dos presuntos, conforme observado em estudos anteriores (TOLDRÁ, RICO e FLORES, 1993). E os tratamentos com substituição de NaCl por KCl apresentaram uma maior quantidade de catepsina B e B+L. No entanto, não foi observada diferença significativa quanto aos aminoácidos presentes no produto, o que demonstra semelhança na atividade da aminopeptidase entre os tratamentos. Dos Santos et al. (2015) não observaram diferença no perfil de eletroforese de proteínas miofibrilares e sarcoplasmáticas, bem como na

composição de aminoácidos livres durante o processo de fabricação de salames com 50% de substituição de NaCl por KCl.

A influência da substituição de NaCl por KCl em níveis de 40 e 70% sobre a geração de compostos voláteis durante a fabricação de lombos suínos curados demonstrou que a substituição de 40% não afetou os compostos voláteis ao final do processamento. Por outro lado, a substituição de 70% de NaCl por KCl não foi indicada pois um maior número de compostos provenientes da oxidação lipídica foram formados, o que pode indicar uma perda de qualidade de aroma e sabor dos lombos suínos curados (WU et al., 2015).

Neste contexto, o fator limitante do uso de KCl em produtos cárneos é seu gosto residual amargo (ASKAR, EL-SAMAHY, TAWFIK, 1994; GUÀRDIA et al., 2008) já que a maioria dos estudos demonstram que a substituição de NaCl por KCl em níveis de até 50% não interferem no processo tecnológico e de estabilidade dos produtos. Segundo Terrell (1983) e Pasin et al. (1989), misturas de cloreto de sódio e cloreto de potássio (50:50) em solução causaram o aumento do sabor metálico, perda do gosto salgado e adstringência. Diversos estudos relatam a baixa aceitação sensorial de produtos cárneos fermentados com substituição de NaCl por KCl. Gou et al. (1996) concluíram que níveis acima de 40% de substituição de NaCl por KCl causaram uma depreciação da qualidade sensorial de lombo suíno curado e salames. Por outro lado, Armenteros et al. (2009) não encontraram diferença nas características sensoriais de presunto cru com substituição de 50% de NaCl por KCl.

Um estudo sensorial sobre a atitude do consumidor diante de produtos cárneos com reduzido teor de NaCl e aceitação de salames com substituição de 50% de NaCl por KCl foi desenvolvido por Guàrdia et al. (2006). Os resultados indicaram que as mulheres possuem uma atitude positiva em relação aos

produtos cárneos com redução de NaCl. E os salames com redução de 50% de NaCl (11g/Kg) e com adição de 50% de KCl tiveram uma boa aceitação sensorial. Os mesmos autores, estudaram a substituição molar de 50% de NaCl por KCl em salames e observaram uma boa aceitação global dos produtos reformulados independente do sexo, idade, escolaridade e nível social (GUÀRDIA et al., 2008).

A qualidade sensorial de salames com substituição de 50% de NaCl por KCl foi estudada por Campagnol et al. (2011b) que encontraram um decréscimo da qualidade sensorial dos salames apesar de não haver alterações no processo de fabricação. Corral et al. (2013) não encontraram diferença na qualidade sensorial de salames produzidos com 2,26% de NaCl e 0,43% de KCl em comparação com o salame tradicional (2,70% de NaCl), exceto para o aroma que foi prejudicado pela adição de KCl. Recentemente, Santos et al. (2014) relataram que a substituição de NaCl por KCl não deve ultrapassar 50% para que as propriedades sensoriais sejam semelhantes ao produto tradicional.

Na tentativa de minimizar os defeitos sensoriais provocados pela adição de KCl nos produtos cárneos fermentados, o uso de realçadores e mascaradores de sabor tem sido estudado. A aplicação de glicina (GOU et al., 1996; GELABERT et al., 2003) e lactato de potássio (GELABERT et al., 1995; 2003) foi utilizada em embutidos fermentados como uma alternativa para melhorar a qualidade sensorial dos produtos com substituição de NaCl por KCl. No entanto, estes compostos não foram capazes de suprimir os defeitos sensoriais desenvolvidos pelo uso de KCl em elevadas concentrações. Recentemente, o uso de aminoácidos (lisina e taurina) e realçadores de sabor 5’ ribonucleotídeos (glutamato monossódico, inosinato dissódico e guanilato dissódico) mostraram ser eficientes para suprir os defeitos sensoriais provocados pela substituição de

NaCl por KCl em níveis de 50 e 75% em embutidos fermentados (CAMPAGNOL et al., 2012; CAMPAGNOL et al., 2012a; SANTOS et al., 2014). De forma semelhante, a adição de extrato de levedura na concentração de 1 e 2% foi eficiente para manter as propriedades sensoriais de salames com substituição de 50% de NaCl por KCl (CAMPAGNOL et al., 2011b).

6.2 Substituição Parcial de NaCl por sais divalentes

A aplicação de sais divalentes em produtos cárneos vem sendo utilizada na forma de mistura de sais, onde a redução de NaCl acompanha uma mistura de sais mono e divalentes. Estes sais têm seu uso limitado porque podem alterar o processo tecnológico de fabricação, estabilidade microbiológica e afetar negativamente as características sensoriais dos produtos. Por outro lado, a adição de sais como $CaCl_2$ e $MgCl_2$ podem trazer benefícios a saúde dos consumidores. O cálcio possui inúmeras funções fisiológicas no organismo, entre elas a formação da massa óssea, regulação da pressão arterial e cardíaca, enquanto o magnésio auxilia na absorção de minerais, controla o metabolismo de carboidratos, lipídeos e proteínas, entre outros (CARVALHO, 1999). Além disso, apesar das vantagens tecnológicas apresentadas pelo KCl, seu uso exclusivo como sal substituto pode resultar num excesso de ingestão de potássio, com risco de ocorrência de hiperpotassemia (THEISEN-TOUPAL, 2015).

Desta forma, a utilização destes sais divalentes em produtos cárneos fermentados mostra-se como uma boa alternativa para a modificação do conteúdo mineral destes produtos. Gimeno, Astiasarán e Bello (1998) utilizaram uma mistura contento 1% de NaCl, 0,55% de KCl, 0,23% de $MgCl_2$ e 0,46% de $CaCl_2$ para substituir o teor de NaCl em salames. Os resultados indicaram que a

mistura de sais modificou o processo de fermentação e maturação dos salames, ao aumentar os valores de pH e atividade de água. A contagem microbiológica de bactérias láticas não teve influência da mistura de sais, enquanto que as *Micrococaceas* tiveram uma diminuição de suas contagens. Além disso, menor aceitação sensorial, devido a diminuição do gosto salgado dos salames com adição da mistura de sais foi percebida pelos consumidores. Em outro estudo, os pesquisadores avaliaram em embutido fermentado tipo espanhol (*Chorizo de Pamplona*) o efeito da mistura de 1% de NaCl, 0,55% de KCl e 0,74% de $CaCl_2$ e foi constatado uma alteração do perfil de textura destes produtos com redução da dureza, coesividade, gomosidade e mastigabilidade. Além disso, os valores instrumentais de cor L* e b* foram aumentados pela adição da mistura de sais. Na avaliação sensorial os salames foram considerados aceitáveis para os atributos de cor e textura, apesar das baixas notas (GIMENO, ASTIASARÁN e BELLO, 1999). A fim de complementar o estudo acima, Gimeno, Astiasarán e Bello (2001) avaliaram a estabilidade microbiológica dos salames produzidos com 1% NaCl, 0,55% KCl e 0,74% $CaCl_2$. Em comparação com o tratamento controle (2,6%NaCl), não foi observada nenhuma diferença na contagem de bactérias láticas e micrococos, o que demonstra que esta mistura de sais manteve a segurança e qualidade microbiológica dos embutidos fermentados. Blesa et al. (2008) obtiveram resultados similares ao avaliarem em presunto cru a mistura de 50% NaCl e 50% KCl e 55% NaCl, 25% KCl, 15% $CaCl_2$ e 5% $MgCl_2$.

Em salames, a combinação dos sais KCl, $CaCl_2$ e $MgCl_2$ não afetou as propriedades físico-químicas de pH, atividade de água e tempo de secagem (ZANARDI et al., 2010). Porém, foi verificado um decréscimo na qualidade sensorial com alterações na intensidade da cor, salinidade e aceitação global

dos salames (ZANARDI et al., 2010). De acordo com Zanardi et al. (2010) estão os resultados encontrados por Aliño et al. (2010), que não encontraram diferença nas características físico-químicas e microbiológicas para lombos suínos curados com substituição de até 45% de NaCl por 25% de KCl, 15% de $CaCl_2$ e 5% de $MgCl_2$. Neste mesmo estudo, a substituição de 70% de NaCl prejudicou o perfil de textura dos lombos suínos curados, ao resultar no significativo aumento da dureza e mastigabilidade.

Além das características físico-químicas e microbiológicas, a redução ou substituição de NaCl por sais divalentes pode interferir nas reações de lipólise e proteólise. Ripollés et al. (2011) estudaram ao longo do processo de fabricação de presunto cru, a interferência da substituição de NaCl por outros sais de cloreto (KCl, $CaCl_2$ e $MgCl_2$) sobre as reações de lipólise e oxidação lipídica. Os resultados demonstraram que a lipólise foi maior no tratamento com 55% NaCl, 25% KCl, 15% $CaCl_2$ e 5% $MgCl_2$ ao produzir durante o processamento um aumento no teor de ácidos graxos livres (RIPOLLÉS et al., 2011). O efeito da substituição parcial de NaCl por uma mistura de sais de cloreto (NaCl, KCl, $MgCl_2$ e $CaCl_2$) sobre as reações proteolítica foi avaliada em presunto cru (ARMENTEROS et al., 2012). Os autores observaram que ao final do processo de fabricação não foi reportada nenhuma alteração nos fenômenos de proteólise, uma vez que a liberação de aminoácidos livres não foi afetada. No entanto, todos os atributos sensoriais foram prejudicados pela adição da mistura de sais nos presuntos crus. Em salames, Dos Santos et al. (2015) encontraram diferença no perfil de eletroforese das proteínas sarcoplasmáticas e um aumento da geração de aminoácidos livres durante o processo de fabricação para os salames produzidos com 50% de NaCl e 50% de $CaCl_2$ e *blend* de 50% de NaCl, 25% de KCl e 25% de $CaCl_2$.

7 Considerações Finais

Observa-se a partir da revisão bibliográfica o quanto é importante ter uma visão geral do consumo de NaCl em diferentes países e as medidas que estão sendo adotadas pelas entidades regulatórias e órgãos de saúde pública, e a partir deste ponto, entender as funções do NaCl no processamento de embutidos fermentados para que a redução ou substituição de NaCl nestes produtos seja promissora. Sendo assim, pode-se observar que existe a necessidade de um estudo mais aprofundado dos parâmetros relacionados às reações bioquímicas que ocorrem durante o processo de fabricação e vida de prateleira de salames. Além disso, a qualidade sensorial precisa ser mais explorada a fim de gerar um perfil sensorial dos produtos reformulados e desta forma tentar minimizar os defeitos sensoriais provados pela redução ou substituição parcial de NaCl por outros sais de cloreto como KCl, $CaCl_2$ e $MgCl_2$.

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CAPÍTULO 2

IMPACT OF SODIUM CHLORIDE REPLACEMENT BY SALT SUBSTITUTES ON THE PROTEOLYSIS AND RHEOLOGICAL PROPERTIES OF DRY FERMENTED SAUSAGES

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Abstract

The effect of a 50% reduction of NaCl and its replacement by KCl, $CaCl_2$, and a blend of KCl and $CaCl_2$ (1:1) on the proteolysis and rheological properties of dry fermented sausages was investigated. The reduction or replacement of NaCl by KCl did not cause changes in the electrophoretic profile and the addition of $CaCl_2$ decreased the degradation of sarcoplasmic proteins during the manufacturing process. Samples with a 50% reduction of NaCl showed a higher content of the amino acids Arg, Glu, His, Val, Cys, Lys, and Trp, whereas the samples containing $CaCl_2$ had a higher content of the amino acids Asp, Thr, Ala, Met, Leu, Ile, and Phe. The reduction or replacement of NaCl by KCl decreased sample firmness, whereas the addition of $CaCl_2$ increased the hardness of the samples.

Keywords: proteolysis, free amino acids, rheology, sodium chloride, potassium chloride, calcium chloride.

1 Introduction

A diet high in sodium increases the risk of hypertension and the incidence of cardiovascular disease (Desmond, 2006) and is considered a risk factor for other diseases, such as obesity (He and MacGregor, 2010), kidney stones, osteoporosis, and certain cancers (Cappuccio et al., 2000). According to the World Health Organization (WHO, 2010), meat products contribute approximately 20–30% of the sodium in a person's diet. Thus, reducing sodium in these products may be useful to help people reduce their sodium intake by up to 2 grams, as recommended by governmental authorities (WHO, 2010).

Dry fermented sausages are among the meat products that have the highest NaCl content, which can reach as high as 6% (Campagnol et al., 2011). Because these products are consumed worldwide, several studies have evaluated the effect of sodium reduction on their physical chemical and biochemical (Gelabert et al., 2003, Flores et al., 2006, Campagnol et al., 2011, Dos Santos et al., 2014, Aaslyng et al., 2014).

Despite sodium reduction being widely covered in the scientific literature, the effects of the reduction or replacement of NaCl by other chloride salts on the proteolysis reactions that occur during the manufacturing process of dry fermented sausages remain minimally studied. These reactions are very important to the quality of the final product, as low-molecular-weight compounds are formed during proteolysis, including peptides and free amino acids, which have a great impact in flavor and texture development (Toldrá, 2008), in addition to being important precursors to the formation of aroma compounds (Toldrá, 2006a). Proteolysis is a result of the action of tissue and microbial enzymes, whose activity is altered by processing conditions, low water activity, and

especially by sodium chloride, because this ingredient regulates the activity of proteolytic enzymes, inhibiting the enzyme activity when its concentration increases during the drying stage (Toldrá, 2002). Thus, the reduction of NaCl in a formulation may increase the activity of proteolytic enzymes, resulting in a more soft and brittle texture due to the higher degradation of myofibrillar proteins (Toldrá, 2006b).

Another important issue that has received little attention thus far is the effect of the reduction or replacement of NaCl by other chloride salts on the rheological properties of dry fermented sausages. Although some authors have reported that salt reduction may interfere with the texture (Gimeno et al., 2001, Dos Santos et al., 2014), rheological properties in uniaxial compression and creep tests during the storage of dry fermented sausages have not been explored. The uniaxial compression and creep tests are fundamental tests to evaluate viscoelastic properties, and have been widely used in the rheological studies of food products. They permit a rapid characterization of the material behavior (Gunasekaran and Ak, 2002) and provide information on the permanent cross-linking of the protein network structure (Fox et al., 2000 Andrés et al., 2008). Thus, these tests can help to elucidate the texture changes in low-sodium dry fermented sausages. This study aimed to investigate the effect of a 50% reduction of NaCl, or a 50% replacement of NaCl by KCl, $CaCl_2$, and a blend of KCl and $CaCl_2$ (1:1) on proteolysis and rheological properties of dry fermented sausages. The myofibrillar and sarcoplasmic proteins and free amino acids were determined along the processing and the rheological properties (texture profile, uniaxial compression and creep compliance tests) were determined during the storage of dry-fermented sausages.

2 Materials and methods

2.1 Dry fermented sausage processing

The formulations were prepared with a 50% reduction of NaCl or a 50% replacement by KCl, $CaCl_2$, or a blend of KCl and $CaCl_2$ (1:1), as shown in Table 1. The dry fermented sausages were produced using the following ingredients: pork (650 g/kg; 76.08 ± 0.26% moisture; 18.00 ± 0.82% protein; 4.43 ± 0.10% lipids; 1.07 ± 0.61% ash), beef (200 g/kg; 77.12 ± 0.22% moisture; 18.57 ± 0.12% protein; 2.20 ± 0.02% lipids; 1.01 ± 0.61% ash), and pork back fat (150 g/kg; 17.28 ± 1.24% moisture; 6.54 ± 0.94% protein; 75.39 ± 0.51% lipids; 0.36 ± 0.55% ash). The raw material was ground with a disk (8 mm) and mixed with the respective amount of NaCl and other ingredients for each treatment as described in Table 1. The following ingredients were added to the meat mixture in each treatment: glucose (5 g/kg), sucrose (5 g/kg), sodium nitrate (0.15 g/kg), sodium nitrite (0.15 g/kg), sodium ascorbate (0.25 g/kg), white pepper (2 g/kg), garlic (3 g/kg), nutmeg (0.02 g/kg) and starter culture (0.25 g/kg; SPX Floracarn, Chr Hansen). After complete homogenization, the treatments were stuffed in collagen casings (diameter of 60 mm), and they were cut into slices of approximately 15 cm in length. In total, 60 pieces (approximately 300 g each) were prepared for each treatment. After being stuffed, the samples were subjected to a bath containing a 20% solution of potassium sorbate, and the samples were then ripened in a laboratory ripening cabinet (Menoncin, Erechim, Brazil). The temperature and relative humidity (TºC/RH%) were set as follows: first day, 25°C/95%; second day, 24°C/93%, third day, 23°C/90%, fourth day, 22°C/85%, fifth day, 21°C/80%, sixth day, 20°C/75%, and from the seventh day to the

nineteenth day, 18°C/75%. The air speed remained constant at 5 m/s throughout the processing.

Table 1. Levels of sodium chloride, potassium chloride, and calcium chloride used in dry fermented sausage formulations.

	Treatments (%)				
	Control	**F1**	**F2**	**F3**	**F4**
Sodium chloride (NaCl)	2.5	1.25	1.25	1.25	1.25
Potassium chloride (KCl)	-	-	1.25	-	0.625
Calcium chloride ($CaCl_2$)	-	-	-	1.25	0.625

* Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl, 25% KCl and 25% $CaCl_2$.

2.2 Proteolysis analysis

The proteolysis in the dry fermented sausages was determined in triplicate throughout the manufacturing process (0, 7, and 19 days). The extraction of myofibrillar and sarcoplasmic proteins was performed according to the methodology described by Díaz et al. (1997), with some modifications. Accordingly, 5 g samples were homogenized with a 35 mL phosphate buffer (0.03 mol/L, pH 7.4) for 2 min at 4 °C using an Ultra Turrax (13,500 rpm). Then, the sample was centrifuged at 10,000g for 20 minutes at 4 °C. The supernatant containing the sarcoplasmic proteins was filtered through glass wool and kept in refrigeration (4 °C ± 1 °C). The pellet was washed twice under the conditions described above to remove any remaining sarcoplasmic proteins. The resulting pellet was homogenized with a 25mL solution of 8 mol/L urea and 1% β-mercaptoethanol for 2 min at 4 ° C using an Ultra Turrax (13,500 rpm), followed by centrifugation at 10,000g for 20 minutes at 4 °C. The supernatant containing the myofibrillar proteins was filtered through glass wool, and kept in refrigeration (4 °C ± 1 ºC) until analysis.

The concentration of myofibrillar and sarcoplasmic proteins was determined by the method of Bradford (1976) using bovine serum albumin (Sigma–Aldrich, USA) as a protein standard.

The proteolysis was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (Laemmli, 1970) using a Mini Protean II apparatus (Bio Rad, CA, USA) and 8% and 17.5% polyacrylamide gradient gels. The samples (4 mg/mL) were dissolved in a sample buffer (2% SDS and 5% β-mercaptoethanol) and heated at 96 °C for 10 min. An aliquot of 10 μL (4 mg of protein/mL) was loaded onto the gels. The gels were stained using 0.1% Coomassie Blue, and destained in a solution of acetic acid: methanol: distilled water (1:4:5). A 10,000 to 250,000 relative molecular masses (M_r) marker kit (Precision Plus Protein Kaleidoscope Standards, Biorad, USA) was used for the standard. The molecular weights of the products of proteolysis were estimated based on the relative mobility of the protein standards.

2.3 Determination of free amino acids

The free amino acids in the dry fermented sausages were determined at 0, 7, and 19 days after the manufacturing process. Samples were triturated (Retsch, USA), and 5 g were homogenized with 0.1 mol/L HCl 1:5 (w/v) for 30 minutes using an orbital shaker. The supernatant was filtered using Whatman filter paper No. 1 (1001125), and a 5 mL filtrate was passed through a filter membrane with 0.45μm pores (HPLC grade). Then, a 1mL aliquot was transferred to a glass tube (6 mm x 50 mm) for derivatization using a phenyl isothiocyanate (PITC) reagent according to the method described by Hagen et al. (1989). Amino acids were determined by RP-HPLC using a Shimadzu HPLC

system (Shimadzu Corporation, Tokyo, Japan) equipped with a UV detector (254 nm) and a C18 column Luna-Phenomenex (250 mm x 4.6 mm, 5µ) (Phenomenex Inc., Torrence, CA, USA). The identification of amino acids was performed by the external standard method (Pierce/ PN 20088), and quantification was performed as described by White et al. (1986) and Hagen et al. (1989).

2.4 Texture measurements

Texture profile analysis (TPA) was performed at the end of the manufacturing process and every 30 days during refrigerated storage (4°C ± 1°C) for 90 days using the TA-XT21 Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK) with a load cell of 25 kg. Each sample was cut into 3 cm cylinders, and stretched axially into two consecutive circles compressed at 30% with a probe of 30 mm in diameter, moving at a constant test velocity of 1 mm/s. The data were collected, and the texture profile curves were drawn using the program Texture Expert, version 1.11 (Stable Micro Systems Ltd.). The following texture parameters were calculated: hardness, springiness, cohesiveness, resilience and chewiness. For each treatment, 5 pieces of dry fermented sausages were used in the instrumental texture analysis.

2.5 Rheological measurements

The viscoelastic behavior of the dry fermented sausage formulations was evaluated after the manufacturing process (day 0), and at 30, 60 and 90 days of refrigerated storage (4ºC ± 1ºC). The formulations were intended to reduce and/or partially replace the amount of sodium in dry fermented sausages. The

rheological analyses were performed using a TA-XT21 Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK), fitted with a 25 kg load cell. The texture analyzer was equipped with a fixed platform and an aluminum plate 35 mm in diameter. No lubrication of the plate or platform was required because the exudate formed during the test was sufficient to reduce the friction between the sample surface and the plate/platform to insignificant levels. The samples were cut into cylinders (25 mm in diameter, 20 mm in height), wrapped individually in PVC film and placed in waterproof plastic bags, which were then maintained in a refrigerated bath at 10 °C for at least 15 min before testing. All rheological analyses were performed at least five times.

2.5.1 Uniaxial compression test

The uniaxial compression was performed according to the Karlsson et al. (2007) methodology. The breakdown pattern of the dry fermented sausage samples was observed by compressing the samples to 20% of their initial sample height at a cross-head speed of 1 mm s^{-1}. Force-displacement data were converted to true stress (σ_t) (Eq. 1) and true strain (ε_t) (Eq. 2) as described by Wium and Qvist (1997).

$$\sigma_t = \frac{F(t)}{A(t)} = \frac{F(t)H(t)}{A_0 H_0} \quad (1)$$

$$\varepsilon_t = \left| \ln\left(\frac{H(t)}{H_0} \right) \right| = \left| \ln\left(\frac{H_0 - \Delta H}{H_0} \right) \right| \quad (2)$$

where $F(t)$, $A(t)$ and $H(t)$ are the applied force, cross-sectional area and displacement at any time, respectively; A_0 and H_0 are the initial sample cross-sectional area and initial sample height, respectively; and ΔH is the absolute deformation.

The following parameters were calculated from the true stress-true strain curve (σ_t-ε_t): Young's modulus (E); fracture stress (σ_f); fracture strain (ε_f); fracture work (W_f); and maximum stress (σ_{max}) (Wium and Qvist, 1997). The fracture point corresponds to the first local maximum point ($d\sigma/dt = 0$) of the data, where the σ_f and ε_f values are obtained. Young's modulus was calculated as the angular coefficient from the linear regression of the σ_t-ε_t data until the fracture point. The fracture work corresponds to the total energy of the fracture represented by the area behind the curve until the fracture point. The maximum stress was determined as the true stress at maximum compression (80%) (Fox et al., 2000).

2.5.2 Creep compliance test

The creep test was performed according to Chattong et al. (2007) by measuring the deformation from applying a constant force of 0.89 N for 180 s. Then, the force was removed and the sample recovery was measured for an additional 180 s. The force-displacement data were converted to true stress (σ_t) (Eq. 1) and strain (ε) (Eq. 3).

$$\varepsilon = \frac{H(t)}{H_0} \quad (3)$$

where $H(t)$ is the height at any time and H_0 is the initial height of the samples.

The results were expressed as the ratio between the strain measured and the initial stress applied, referred to as compliance ($J(t) = \gamma(t) / \sigma_0$). The experimental compliance data during the creep phase was fitted by the four-component Burgers model, which is expressed in Eq. 4 (Burgers, 1935).

$$J(t) = J_0 + J_1\left(1 - e^{-t/\tau}\right) + \frac{t}{\eta_N} \quad (4)$$

where $J(t)$ is the creep-recovery compliance as a function of time; J_0 is the instantaneous compliance of the Maxwell spring; J_1 is the viscoelastic compliance that represents the retarded compliance related to the Kelvin-Voigt element; τ is the mean retardation time associated to the Kelvin-Voigt element; and η_N is the Newtonian viscosity associated to the Maxwell dashpot.

2.6 Statistical Analysis

Three independent manufacturing processes were performed using the same formulation and technology. For each manufacturer, three sample units (dry fermented sausages) were taken per sampling day (n = 9). All analyses were performed in triplicate. The results reported in this study are the mean obtained from all data recorded for each parameter.

An analysis of variance (one-way ANOVA) and Tukey's test at $P < 0.05$ were used to verify the statistical significance of the results using the statistical software XLSTAT v.5.02 (Addinsoft, Paris, France). The commercial software Statistica v.8 (Statsoft, Inc., Tulsa, OK, USA) was used for a linear and non-linear regression analysis to estimate the model parameters of the uniaxial compression and creep compliance tests, respectively.

3 Results and discussion

3.1 Effect of reduction or replacement of NaCl on proteolysis reactions

The myofibrillar and sarcoplasmic proteins were extracted from the dry fermented sausages with NaCl reduced by 50% and/or replaced by KCl and/or $CaCl_2$. There were no differences in the behavior of the myofibrillar proteins during the manufacturing process (Fig. 1). The proteins myosin (200,000 M_r) and actin (45,000 M_r) showed a severe degradation during the manufacturing process for all treatments. This behavior is normal and expected (García de Fernando and Fox, 1991), and the evidenced proteolytic activity is characteristic of fermented meat products, which involve the release of free amino acids and polypeptides (Sun et al., 2009). After seven days of manufacture, an increase in the number of bands with a molecular weight between 50,000 and 75,000 Mr and 10,000 and 37,000 M_r was observed, which remained until the end of the manufacturing process (19 days). According to Toldrá (1998), the fermentation process of dry fermented sausages provides an increase in the degradation of myofibrillar proteins due to pH values being lower than 5.00. This is the case of myosin and actin proteins, which are degraded into fragments of 135,000 and 38,000 M_r, respectively. Other protein fragments are also formed, with molecular weights of 29,000 and 13,000 M_r. This fact has been reported in other studies on the proteolysis of dry fermented sausages (Aro et al., 2010; Ikonić et al., 2013) and ham (Flores et al., 2006). Other authors have found bands with a molecular weight less than 150,000 M_r during the manufacturing process of dry fermented sausages (Hughes et al., 2002).

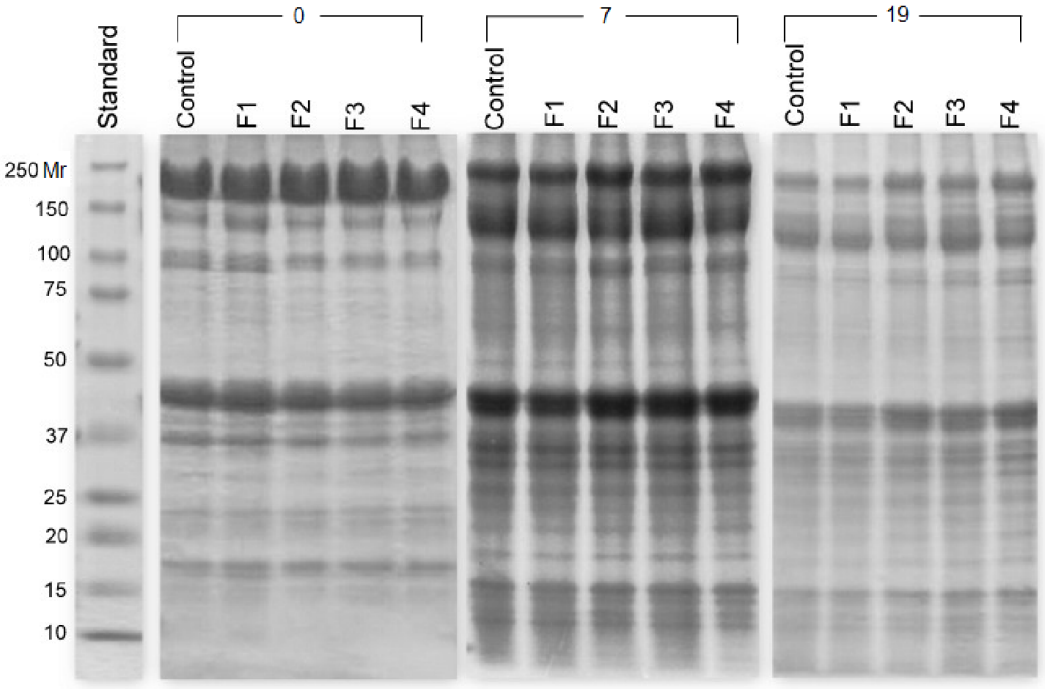

Fig. 1. 8 and 17.5% SDS-PAGE gels of myofibrillar proteins in the dry fermented sausages submitted to five types of salt treatments. Standards: BioRad molecular weight standards (Mr= 250,000 a 10,000). Treatments as described in Table1.

The bands with a molecular weight between 15,000 and 100,000 M_r indicated the presence of sarcoplasmic proteins in dry fermented sausages throughout the manufacturing period (Fig. 2). Polypeptides with a molecular weight between 37,000 and 70,000 Mr and 10,000 and 30,000 M_r were formed from the seventh day of manufacture. Sun et al. (2011) reported that the degradation of high-molecular weight proteins might be due to proteolysis or the manufacturing process leading to pH reduction, increased salt concentration, and the dehydration of dry fermented sausages. This behavior continued until the end of the manufacturing process and was similar to that found by other authors on the proteolysis in dry fermented sausages (García de Fernando and Fox, 1991, Hughes et al., 2002, Sun et al., 2011).

The reduction of NaCl and the addition of $CaCl_2$ altered the electrophoretic profile of the sarcoplasmic proteins during the manufacturing process. On day 0, the bands near Mr 90,000, 45,000, 35,000 and 15,000 appeared with low intensities in the treatments containing $CaCl_2$ (F3 and F4). On day 7, the treatments F3 and F4 also presented lower bands with molecular weight near Mr 25,000.

At the end of the process (19 days), bands near Mr 25,000 were observed with low intensities in the control sample, and in the treatments F1 (50% NaCl) and F2 (50% NaCl and 50% KCl), and reappeared in the treatments F3 (50% NaCl and 50% $CaCl_2$) and F4 (50% NaCl, and 25% KCl and $CaCl_2$ 25%). The lower degradation of sarcoplasmic proteins observed in the samples containing $CaCl_2$ may be due to the slower growth of lactic acid bacteria in these treatments (data not shown), since these microorganisms have shown good ability for protein degradation (Fadda et al., 1999).

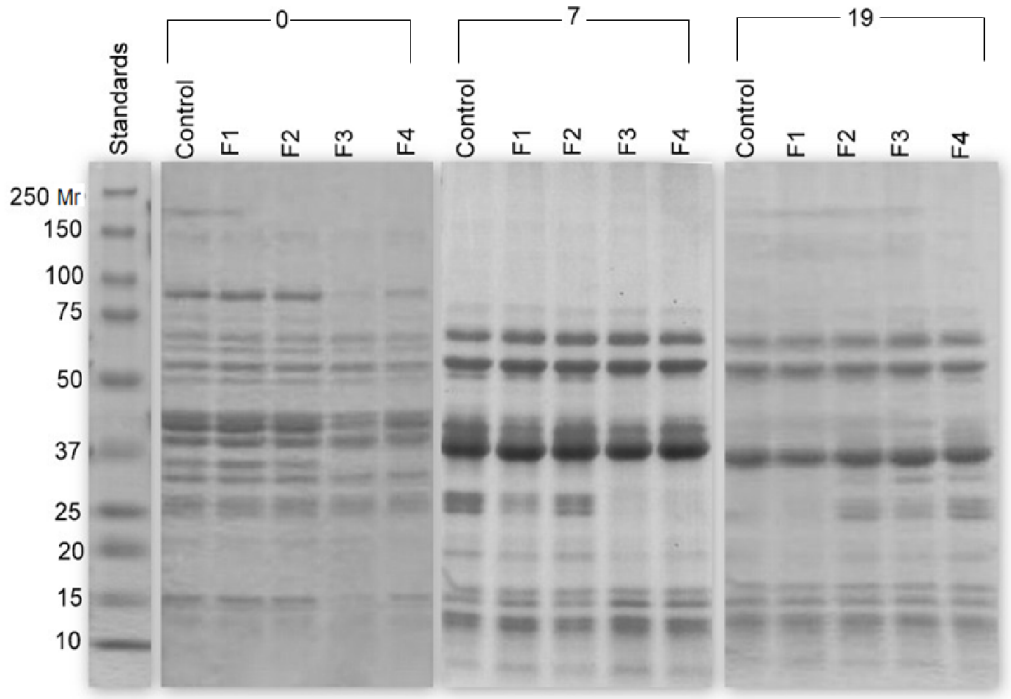

Fig. 2. 8 and 17.5% SDS-PAGE gels of sarcoplasmic proteins in the dry fermented sausages submitted to five types of salt treatments. Standards: BioRad molecular weight standards (Mr= 250,000 a 10,000). Treatments as described inTable1.

The total free amino acids released during the manufacturing of fermented meat products, including dry fermented sausages, depends on the decrease in pH, the salt concentration, the addition of a starter culture and the processing conditions (time, temperature and lower water activity), as these parameters mainly affect the activity of aminopeptidase enzymes (Sanz and Toldrá, 2002). In our study, an increase in the total free amino acids was observed during the manufacturing process (Table 2) for all treatments, and a 50% replacement of NaCl with KCl and/or $CaCl_2$ did not hinder the release of free amino acids. The change occurred during fermentation and ripening process indicating that the highest enzymatic activity took place during these stages (Verplaetse et al., 1989). Several authors have reported a major release of free amino acids at the

beginning of the process in coincidence with the fermentation stage (Díaz et al., 1997, Aro et al., 2010).

In this study, dry fermented sausages produced with a 50% NaCl reduction (F1), containing 1.25% NaCl, had the highest amount of total free amino acids (2230.7 mg / 100 g), which may be due to the reduced salt content. Within the literature there are reports that reduced sodium chloride levels (<2%) may activate most of the muscle proteases, which would increase the proteolytic activity and, therefore, the release of free amino acids (Toldrá, 1992). The treatments with KCl and/or $CaCl_2$ had a similar amount of total free amino acids from the control. Similarly, Armenteros et al. (2012) partially replaced NaCl with a blend of chloride salts (KCl, $CaCl_2$, and $MgCl_2$) in ham and found no difference in the total free amino acids. Also, no difference was observed in the total free amino acids in cured loins prepared with a partial replacement of NaCl by KCl (Armenteros et al., 2009).

The concentration of all free amino acids increased over the 19 days of manufacture, except glycine (Gly) and tyrosine (Tyr), which decreased on the seventh day and increased at the end of the manufacturing process. The decrease in the concentrations of amino acids may indicate that their metabolism by bacteria was more intense than their production during the latter stages of fermentation (Ordóñez et al. 1999, Hughes et al. 2002). Of the 18 free amino acids, a higher arginine concentration was observed in all treatments, and the samples prepared with 50% NaCl (F1) differed significantly from the control. According to Toldrá (2002), aminopeptidases are activated in the presence of salt, and may contribute to the generation of free amino acids in the ripening processes, including arginine. It is worth mentioning that this amino acid is

responsible for the flavor development that is characteristic of dry-cured meat products. No significant difference was observed between the treatments and control for the concentration of the free amino acids serine (Ser), glycine (Gly), and tyrosine (Tyr). In contrast, aspartic acid (Asp), threonine (Thr), alanine (Ala), methionine (Met), leucine (Leu), isoleucine (Ile), and phenylalanine (Phe) concentrations were higher than the control ($P \leq 0.05$) in the treatments with 50% NaCl (F1), 50% NaCl and 50% $CaCl_2$ (F3), and 50% NaCl, 25% KCl, and 25% $CaCl_2$ (F4). This result can have a negative impact on the sensory quality, as high concentrations of Met, Leu and Ile may increase the bitter taste of dry fermented sausages (Toldrá, 2006a). In contrast, higher concentrations of the free amino acids glutamic acid (Glu), histidine (His), valine (Val), cysteine (Cys), lysine (Lys) and tryptophan (Trp) were observed in the treatment with a 50% reduction of NaCl (F1), which differed significantly from the control.

Table 2. Free amino acids content during the manufacturing process of dry fermented sausages with 50% reduction of NaCl, and replacement with KCl and / or $CaCl_2$.

		Treatments					
Free amino acids (FAA) (mg/100g)	**Days**	**Control**	**F1**	**F2**	**F3**	**F4**	**SEM***
Asp	**0**	4.0^{aC}	4.0^{aC}	3.0^{bC}	2.0^{cC}	4.7^{aC}	0.3
	7	18.0^{bB}	13.0^{cB}	15.4^{cB}	22.0^{aB}	35.0^{aB}	0.9
	19	35.0^{cA}	45.3^{bA}	35.0^{cA}	46.0^{bA}	58.0^{aA}	2.3
Glu	**0**	16.0^{cC}	19.7^{aC}	13.0^{eC}	14.3^{dC}	17.7^{bC}	0.7
	7	86.0^{cB}	99.0^{aB}	92.0^{bB}	89.7^{bB}	98.0^{aB}	1.4
	19	171.0^{eA}	199.3^{aA}	178.0^{dA}	185.3^{cA}	192.3^{bA}	2.7
Ser	**0**	8.7^{bC}	10.7^{aC}	8.3^{bC}	8.0^{bC}	9.0^{bC}	0.3
	7	21.3^{bB}	21.7^{bB}	21.3^{bB}	20.7^{bB}	23.4^{aB}	0.2
	19	51.0^{aA}	53.3^{aA}	44.0^{aA}	32.0^{aA}	50.0^{aA}	3.4
Gly	**0**	17.7^{aA}	17.7^{aB}	15.0^{bB}	17.7^{aB}	18.3^{aA}	0.3
	7	11.0^{abB}	10.0^{bC}	11.3^{aC}	9.7^{cC}	11.7^{aB}	0.2
	19	17.3^{aA}	20.0^{aA}	16.0^{aA}	23.3^{aA}	20.0^{aA}	1.2
His	**0**	31.0^{abC}	36.3^{aC}	29.0^{bC}	30.7^{abC}	31.0^{abC}	0.7
	7	65.3^{eB}	81.7^{aB}	71.3^{cB}	67.7^{dB}	74.3^{bB}	1.5
	19	135.7^{cA}	179.3^{aA}	135.7^{cA}	135.7^{cA}	148.0^{bA}	4.6
Arg	**0**	246.8^{abC}	231.7^{cC}	207.7^{dC}	244.7^{bC}	249.7^{aC}	4.1
	7	338.0^{dB}	353.0^{bB}	362.0^{aB}	337.7^{dB}	349.7^{cB}	2.5
	19	605.7^{bA}	632.0^{aA}	565.3^{cA}	603.3^{bA}	605.0^{bA}	5.7
Thr	**0**	8.3^{aC}	8.7^{aC}	7.3^{aC}	7.7^{aC}	8.3^{aC}	0.2
	7	22.3^{cB}	25.7^{bB}	24.3^{bB}	28.3^{aB}	29.3^{aB}	0.7
	19	45.0^{cA}	56.0^{aA}	43.7^{cA}	52.0^{bA}	52.0^{bA}	1.3
Ala	**0**	34.8^{cC}	38.7^{aC}	32.3^{cC}	33.7^{cdC}	36.3^{bC}	0.6
	7	77.3^{eB}	91.3^{aB}	83.4^{cB}	81.3^{dB}	87.4^{bB}	1.3
	19	151.7^{dA}	184.3^{aA}	150.3^{dA}	158.7^{cA}	165.7^{bA}	3.3
Pro	**0**	7.7^{abC}	9.0^{aC}	6.7^{bC}	7.3^{bC}	7.7^{abC}	0.2
	7	21.3^{aB}	20.7^{aB}	20.7^{aB}	16.7^{bB}	19.7^{aB}	0.4
	19	38.0^{aA}	37.0^{aA}	35.7^{abA}	28.3^{cA}	33.0^{bA}	1.0
Tyr	**0**	2.0^{aA}	1.0^{aB}	1.3^{aB}	2.0^{aB}	1.4^{aB}	0.1
	7	0.3^{aB}	0.3^{aB}	0.7^{aB}	1.3^{aB}	1.3^{aB}	0.2
	19	3.0^{aA}	5.0^{aA}	2.0^{bA}	5.0^{aA}	5.0^{aA}	0.4
Val	**0**	6.7^{cC}	10.0^{aC}	6.7^{cC}	7.3^{bcC}	8.7^{abC}	0.3
	7	27.3^{dB}	34.3^{aB}	30.3^{cB}	31.7^{bcB}	32.7^{abB}	0.6
	19	52.0^{cA}	67.7^{aA}	56.0^{bA}	56.7^{bA}	58.3^{bA}	1.4
Met	**0**	5.3^{aC}	5.3^{aC}	4.3^{aC}	4.3^{aC}	4.7^{aC}	0.1
	7	11.7^{bB}	15.7^{aB}	13.3^{bB}	15.3^{aB}	15.3^{aB}	0.3
	19	24.3^{cA}	32.0^{aA}	25.0^{cA}	28.7^{bA}	28.7^{bA}	0.8
Cys	**0**	10.3^{abC}	11.7^{aC}	7.3^{cC}	9.4^{bC}	9.7^{bC}	0.4
	7	48.3^{bB}	60.3^{aB}	30.0^{cB}	32.7^{cB}	34.3^{cB}	3.1
	19	126.0^{cA}	155.3^{aA}	107.7^{dA}	124.3^{cA}	135.3^{bA}	4.2
Ile	**0**	6.3^{bC}	8.3^{aC}	6.3^{bC}	6.4^{bC}	8.3^{aC}	0.3
	7	24.3^{bB}	29.7^{aB}	26.7^{bB}	29.7^{aB}	29.7^{aB}	0.6

	19	46.3^{eA}	62.0^{aA}	50.7^{dA}	59.0^{abA}	57.7^{cA}	1.6
Leu	**0**	12.3^{bC}	15.7^{aC}	11.3^{bC}	12.3^{bC}	14.3^{aC}	0.5
	7	42.7^{bB}	68.7^{aB}	55.7^{abB}	68.3^{aB}	67.7^{aB}	1.8
	19	106.7^{eA}	144.7^{aA}	110.7^{dA}	136.7^{bA}	133.0^{cA}	4.0
Phe	**0**	7.3^{bcC}	9.7^{aC}	6.7^{cC}	7.3^{bcC}	8.3^{abC}	0.3
	7	30.3^{cB}	39.7^{aB}	33.3^{bB}	39.3^{aB}	38.7^{aB}	1.0
	19	58.3^{cA}	78.7^{aA}	61.3^{cA}	77.0^{aA}	72.0^{bA}	2.3
Lys	**0**	13.3^{aC}	14.3^{aC}	9.7^{bcC}	8.7^{cC}	11.4^{bC}	0.6
	7	39.3^{cB}	44.7^{aB}	42.7^{bB}	39.3^{cB}	40.7^{cB}	0.6
	19	87.3^{cA}	111.0^{aA}	92.3^{cA}	92.0^{cA}	99.0^{bA}	2.2
Trp	**0**	ND**	6.3^{aC}	ND**	ND**	3.3^{bC}	0.7
	7	70.8^{cB}	77.9^{aB}	77.4^{aB}	67.3^{dB}	74.3^{bB}	1.0
	19	127.0^{dA}	167.8^{aA}	137.3^{cA}	136.0^{cA}	149.0^{bA}	3.7
Total FAA	**0**	438.5	458.8	375.9	423.8	452.8	
Total FAA	**7**	955.5	1087.4	1011.8	998.7	1063.2	
Total FAA	**19**	1881.3	2230.7	1846.7	1980.0	2062.0	

*SEM- Standard error of the mean.
ND** not detected
Averages in the same row followed by the same letter are not significantly different by Tukey's test ($P \geq 0.05$). Averages in the same column followed by the same uppercase letter are not significantly different by Tukey's test ($P \geq 0.05$). Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl, 25% KCl and 25% $CaCl_2$.

3.2 Effect of reduction/replacement of NaCl on rheological properties

3.2.1 Texture profile

The effect of sodium reduction or replacement on the textural properties, such as hardness, springiness, cohesiveness, resilience, and chewiness, of dry fermented sausage samples is shown in Table 3. The reduction and replacement of NaCl did not significantly ($P < 0.05$) affect the texture parameters, except for hardness values, which decreased in the F1 (50% NaCl) formulation. Similar behavior was observed during the shelf life of dry fermented sausage samples for all formulations.

A comparison of the TPA and rheological results of this study with those in the literature was not possible because no study has investigated the effect of the composition of dry fermented sausages on its texture profile and/or

viscoelastic parameters. Therefore, these results were compared with those obtained for sausages (Houben and van't Hooft, 2005, Andrés et al., 2008) and model system meat emulsions (Yilmaz et al., 2012). Springiness denotes how well a product physically springs back after it has been deformed during the first compression (Bourne, 1978). Springiness values were related to the elastic properties of the samples; thus, an increase in the springiness value indicates that elasticity of the sample is enhanced.

In this study, the dry fermented sausages tended to increase their resilience values for all formulations during 30 days of storage with no further significant ($P > 0.05$) modification after that storage time. Cohesiveness, how good the sample retains its structure after compression (Bourne, 1978), tended to increase with time, which is probably due to the effect of fat on cohesiveness. A similar trend was also found in the literature (Papadina and Bloukas, 1999, Saricoban et al., 2009).

Table 3. Texture profile analysis during refrigerated storage of dry fermented sausages with 50% reduction of NaCl, and replacement with KCl and / or $CaCl_2$.

Texture parameter	Storage time (days)	Control	F1	F2	F3	F4	SEM*
Hardness (N)	0	47.35^{abc}	37.11^{c}	58.16^{a}	42.84^{c}	51.38^{ab}	1.90
	30	43.30^{ab}	36.37^{b}	52.46^{a}	37.73^{b}	52.12^{a}	1.65
	60	39.95^{a}	23.52^{b}	39.16^{a}	27.23^{b}	42.17^{a}	1.76
	90	45.74^{a}	27.52^{b}	50.08^{a}	33.71^{b}	48.52^{a}	1.85
Springiness	0	0.64^{ab}	0.66^{a}	0.62^{b}	0.65^{ab}	0.63^{ab}	0.01
	30	0.68^{a}	0.70^{a}	0.64^{a}	0.69^{a}	0.69^{a}	0.02
	60	0.71^{a}	0.65^{a}	0.68^{a}	0.74^{a}	0.70^{a}	0.02
	90	0.70^{a}	0.68^{a}	0.65^{a}	0.66^{a}	0.71^{a}	0.01
Cohesiveness	0	0.61^{a}	0.61^{a}	0.59^{a}	0.60^{a}	0.59^{a}	0.02
	30	0.67^{a}	0.65^{a}	0.67^{a}	0.64^{a}	0.65^{a}	0.02
	60	0.64^{a}	0.59^{a}	0.62^{a}	0.71^{a}	0.64^{a}	0.03
	90	0.65^{a}	0.65^{a}	0.65^{a}	0.64^{a}	0.64^{a}	0.01
Resilience	0	0.175^{a}	0.182^{ab}	0.177^{a}	0.182^{ab}	0.174^{a}	0.20
	30	0.212^{a}	0.211^{a}	0.212^{a}	0.217^{a}	0.214^{a}	0.58
	60	0.206^{ab}	0.216^{a}	0.213^{a}	0.211^{a}	0.210^{a}	0.75
	90	0.211^{ab}	0.185^{a}	0.200^{a}	0.193^{a}	0.210^{ab}	0.35
Chewiness (N)	0	18.28^{ab}	14.96^{b}	21.29^{a}	16.60^{ab}	19.04^{ab}	0.65
	30	19.51^{abc}	16.41^{c}	22.37^{ab}	16.62^{bc}	23.26^{a}	0.75
	60	18.21^{a}	9.08^{a}	17.07^{a}	16.86^{a}	18.97^{a}	1.43
	90	20.71^{a}	12.10^{a}	21.53^{a}	14.35^{a}	21.06^{a}	0.91

* SEM- Standard error of the mean.
Averages in the same row followed by the same letter in lowercase are not significantly different by Tukey test ($P > 0.05$). Control - 100% NaCl; F1 - 50% NaCl; F2 - 50% NaCl and 50% KCl; F3 - 50% NaCl and 50% $CaCl_2$; F4 - 50% NaCl, 25% KCl and 25% $CaCl_2$.

3.2.2 Uniaxial compression test

The values for Young's modulus, fracture stress, fracture strain, work for fracture, and maximum stress calculated for the low-sodium dry fermented sausages during the 90 days of refrigerated storage are shown in Table 4. Young's modulus (E) provides a useful indication of how easily a sample can be contracted and stretched, sometimes referred to as the stiffness of the material, and corresponds to the slope of the linear portion of the σ_t-ε_t curves, where only Hooke's law is valid (Gunasekaran and Ak, 2002, Fox et al., 2000). The fracture

stress (σ_f) is related to sample toughness, whereas the maximum stress (σ_{max}) and the work for fracture (W_f) are directly related to sample firmness and hardness (Fox et al., 2000). The fracture strain (ε_f) denotes the strain needed to cause a rupture point in the sample and may be associated with the crumbliness of the samples (Cunha et al., 2006).

The values for Young's modulus, fracture stress, fracture strain, maximum stress and work for fracture did not decrease significantly ($P > 0.05$) with storage time, demonstrating the preservation of the rigidity of the samples and, consequently, a well-structured peptide matrix during shelf life. Neither sample F3 (50% NaCl and 50% $CaCl_2$) nor F4 (50% NaCl, 25% KCl and 25% $CaCl_2$) presented enough resistance to cause a fracture point after 90 days of storage.

Regarding the formulations of salts, the 50% reduction and/or replacement of the sodium content significantly affected all viscoelastic parameters, except fracture strain, which indicates that there was no difference between the dry fermented sausage formulations with respect to crumbliness. The 50% reduction of NaCl in F1 caused a decrease in the parameters. This behavior indicates a loss of rigidity due to the weaker intermolecular interaction formed in the protein network, which is probably caused by an ion charge reduction. However, the incorporation of potassium in F2 (50% NaCl and 50% KCl) and F4 (50% NaCl, 25% KCl and 25% $CaCl_2$) increased the viscoelastic parameters, demonstrating a symbiotic effect between sodium and potassium in the formulations of the dry fermented sausage, thus resulting in tougher samples. All dry fermented sausage samples exhibited a higher rigidity when compared to low-fat chicken sausage (Andrés et al., 2008), semi-dry fermented sausage (Houben and van't Hooft, 2005) and meat emulsions (Yilmaz et al., 2012). This characteristic could be

associated with a well-structured protein network resulting in a more solid-like and less viscoelastic behavior.

Table 4. Uniaxial compression parameters content during refrigerated storage of dry fermented sausages with 50% reduction of NaCl, and replacement with KCl and / or $CaCl_2$.

Uniaxial compression parameter	Storage time (days)	Control	F1	F2	F3	F4
E (kPa)	0	245.3^{cA}	185.0^{dA}	271.5^{aA}	214.3^{bA}	290.1^{aA}
	30	251.1^{aA}	160.0^{cB}	232.1^{bC}	174.7^{cB}	252.7^{aB}
	60	221.4^{bB}	167.0^{cdAB}	290.6^{aAB}	166.8^{cdB}	175.4^{cC}
	90	212.9^{bB}	167.0^{cAB}	246.9^{aBC}	-	-
σ_f (kPa)	0	319.7^{cA}	154.9^{dA}	430.1^{aA}	228.5^{eA}	347.7^{bA}
	30	297.3^{bB}	115.6^{eB}	345.6^{aB}	168.7^{dB}	227.1^{cB}
	60	237.6^{bC}	155.0^{cdA}	288.0^{aC}	128.0^{dC}	120.9^{deC}
	90	296.3^{aB}	117.7^{cB}	211.6^{bD}	-	-
ε_f (-)	0	1.283^{abA}	1.077^{bA}	1.168^{abAB}	1.073^{bA}	1.073^{bA}
	30	1.287^{abA}	0.778^{cB}	1.644^{abA}	1.053^{bA}	1.074^{bA}
	60	1.158^{abA}	0.977^{abAB}	1.059^{abB}	0.867^{bAB}	0.757^{bAB}
	90	1.358^{abA}	0.977^{bAB}	1.079^{abAB}	-	-
W_f (kJ/m^3)	0	212.6^{bcA}	84.3^{dA}	252.0^{abB}	121.2^{cA}	192.2^{bA}
	30	195.4^{bB}	45.7^{eD}	316.0^{aA}	89.8^{dB}	121.4^{cB}
	60	142.6^{bC}	76.0^{cB}	153.8^{aC}	55.5^{cdC}	46.7^{cdC}
	90	220.8^{aA}	58.0^{cC}	117.8^{bD}	-	-
σ_{max} (kPa)	0	384.8^{cdB}	314.8^{deA}	572.5^{aA}	368.0^{cdB}	469.0^{bA}
	30	410.7^{bA}	268.3^{cB}	438.0^{aB}	289.8^{cC}	438.8^{aA}
	60	326.3^{bC}	324.4^{bA}	326.3^{bC}	404.3^{aA}	257.6^{cC}
	90	404.0^{aA}	271.2^{cB}	334.2^{bC}	334.3^{bB}	375.9^{abB}

$^{a-e}$ Different superscript lowercase letters denote significant differences ($P < 0.05$) among the different studied dry fermented sausages formulations for the same period of storage. $^{A-D}$ Different superscript uppercase letters denote significant differences ($P < 0.05$) among different periods of storage for the same studied dry fermented sausages formulation. Control - 100% NaCl; F1 - 50% NaCl; F2 - 50% NaCl and 50% KCl; F3 - 50% NaCl and 50% $CaCl_2$; F4 - 50% NaCl, 25% KCl and 25% $CaCl_2$.

3.2.3 Creep compliance test

The main characteristic of viscoelastic materials is their continuous deformation when they are exposed to constant tension. The experimental creep compliance data of the dry fermented sausage samples were fitted to a four-component Burgers model ($0.9641 < R^2 < 0.9985$) by non-linear regression. The parameters adjusted are comprised by the instantaneous elastic compliance (J_0), the retarded compliance (J_1), retarted time (τ) and Newtonian viscosity (η_N).

The Levenberg-Marquardt estimation method was used with 5,000 interactions and 10^{-6} least squares as the convergence criterion for the loss function. The adjusted model parameters are shown in Table 5. These parameters indicate some rheological properties, in which the main factor of influence is probably the composition of the dry fermented sausage and processing characteristics. Furthermore, these parameters are good indications not only of the possible texture properties but also of the behavior of the dry fermented sausage throughout the shelf life as they may suffer deformation and changes in composition during storage time.

The formulations significantly influenced ($P < 0.05$) all creep parameters, with the exception of the retardation time (for which $P > 0.05$). The refrigerated storage also significantly ($P < 0.05$) affected these parameters. According to Olivares et al. (2009), the instantaneous elastic compliance (J_0) represents the value of instantaneous shear creep compliance at an initial time, and it may be related to the undisturbed protein network structure (Ma et al., 1997). A higher value of J_0 reflects a higher degree of non-retarded Hookean-type (elastic) deformation, indicating that the polypeptide strands in the network are relatively free to rearrange between cross-links (Ma et al., 1997). In other words, the

instantaneous compliance (J_0) is inversely related to the rigidity of dry fermented sausages (Olivares et al., 2009).

Among the dry fermented sausages studied, the formulation F4 exhibited the lowest J_0 value, followed by F3, F1, the control and F2. This behavior indicates that the $CaCl_2$ addition in F4 and F3 enhanced the dry fermented sausages' rigidity, resulting in more solid-like samples (Lobato-Calleros et al., 2000). By contrast, the use of KCl as a 50% substitute of NaCl in F2 produced softer and less firm samples. These results are probably due to a higher ionic charge of $CaCl_2$, which caused stronger interactions among salt, protein and water molecules fortifying the polypeptide network. An increasing J_0 with storage time indicates that the dry fermented sausages become less rigid. It is worth mentioning that J_0 showed significant differences ($P < 0.05$) with storage time only for the formulations F3 and F4. All dry fermented sausage samples showed very low values of J_0, exhibiting relatively higher rigidity when compared to Thai sausages (Chattong et al., 2007). According to Olivares et al. (2009), the retarded compliance (J_1) represents the principal component of the viscoelastic behavior of dry fermented sausage. It was observed that J_1 significantly ($P < 0.05$) increased with storage time for all formulations. The increase of this parameter is associated with a less solid and more viscoelastic behavior, which confirms the results observed for J_0. In addition, Newtonian viscosity (η_N), which is derived from the slope of the curve at large values of time, may be attributed to the breakdown of protein network structures (Messens et al., 2000). A decrease in η_N was observed with a decrease in sodium concentration for F1 (50% NaCl) and F3 (50% NaCl and 50% $CaCl_2$), indicating a lower resistance to flow. Thus, both formulations show a less solid-like behavior in dry fermented sausage samples.

Table 5. Creep viscoelastic parameters of Burger's model during refrigerated storage of dry fermented sausages with 50% reduction of NaCl, and replacement with KCl and / or $CaCl_2$.

Creep viscoelastic parameters	Storage time (days)	Control	F1	F2	F3	F4
J_0 (10^{-22} Pa^{-1})	0	3.47^{bB}	3.36^{bA}	6.33^{aA}	0.57^{cBC}	0.01^{dA}
	30	5.59^{aA}	3.51^{bA}	2.22^{cD}	2.07^{cA}	0.13^{dA}
	60	0.16^{bC}	0.13^{bC}	3.42^{aC}	0.13^{bC}	0.11^{bA}
	90	3.39^{bB}	2.44^{cB}	4.07^{aB}	1.97^{dB}	0.14^{eA}
J_1 (10^{-5} Pa^{-1})	0	2.75^{bC}	3.36^{aD}	2.66^{bB}	3.58^{aC}	3.13^{abB}
	30	3.52^{bB}	4.77^{aC}	2.12^{cC}	4.72^{aB}	2.07^{cC}
	60	2.24^{dC}	5.61^{aB}	2.83^{dB}	4.26^{bB}	3.49^{cB}
	90	5.51^{bA}	6.91^{aA}	3.53^{dA}	5.88^{bA}	4.42^{cA}
τ (s)	0	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}
	30	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}
	60	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}
	90	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}
η_N (10^7 Pa.s)	0	2.94^{aB}	1.63^{bA}	2.61^{aB}	1.86^{bA}	2.90^{aA}
	30	2.53^{bC}	1.66^{cA}	3.57^{aA}	1.82^{cA}	2.77^{bA}
	60	3.70^{aA}	1.62^{cA}	3.19^{abA}	1.84^{cA}	2.80^{bA}
	90	2.12^{aC}	1.18^{cA}	1.70^{bC}	1.34^{cA}	2.24^{aA}

[a-e] Different superscript lowercase letters denote significant differences ($P < 0.05$) among the different studied dry fermented sausages formulations for the same period of storage. [A-D] Different superscript uppercase letters denote significant differences ($P < 0.05$) among different periods of storage for the same studied dry fermented sausages formulation. Control - 100% NaCl; F1 - 50% NaCl; F2 - 50% NaCl and 50% KCl; F3 - 50% NaCl and 50% $CaCl_2$; F4 - 50% NaCl, 25% KCl and 25% $CaCl_2$.

3.2.4 A comparison of texture and rheological measurements

At first sight, TPA results may appear inconsistent with creep and uniaxial compression tests. However, resilience, a measurement of how the sample recovers from deformation, is the parameter that is able to provide a real indication of the elasticity of a product. Indeed, the best correlation among texture and rheological measurements can be observed between resilience and J_1 values and between resilience and E values, specially with respect to formulations ranging from 0 to 60 days. Resilience is how well a product resists regaining its original shape (Bourne, 1978). Considering the results obtained by

the creep test, this phenomenon may be indicative of the loss of rigidity that unfortunately could not be measured. As mentioned in the creep tests, this likely loss of elasticity may be associated to proteolysis during storage. The reduction and/or replacement of sodium caused significant changes in the firmness, elasticity and texture of the samples compared to the control. The reduction of NaCl in F1 had a relative decreasing effect on the firmness of the dry fermented sausages when compared to the control, whereas the addition of $CaCl_2$ in formulations F3 and F4 caused an increase in the hardness of the dry fermented sausages. In addition, those samples showed pronounced decay in their firmness over storage time.

4 Conclusion

The reduction of 50% NaCl increased the total free amino acids released during the manufacturing and had a relative decreasing effect on the firmness of the dry fermented sausages. The addition of $CaCl_2$ modified the total free amino acids released and decreased the degradation of sarcoplasmic proteins during the manufacturing process. In addition, $CaCl_2$ increased the hardness of the dry fermented sausages and those samples showed pronounced decay in their firmness over storage time. Such changes are undesirable, as they can interfere with the sensory consumer's acceptability and/or preference.

The 50% replacement of NaCl by KCl did not influence the proteolysis and exhibited the most similar viscoelastic properties to the control and a greater stability during storage, demonstrating a potential application of this formulation

in the marketplace. However, sensory analysis should be performed to evaluate consumer acceptability.

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CAPÍTULO 3

GENERATION OF VOLATILE COMPOUNDS IN BRAZILIAN LOW-SODIUM DRY FERMENTED SAUSAGES CONTAINING BLENDS OF NaCl, KCl, AND $CaCl_2$ DURING PROCESSING AND STORAGE

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ABSTRACT

Brazilian dry fermented sausages with different salt content were manufactured: control (2.5% NaCl), 50% salt reduced (1.25% NaCl, F1), 50% replaced by KCl (1.25% NaCl and 1.25% KCl, F2), 50% replaced by $CaCl_2$ (1.25% NaCl and 1.25% $CaCl_2$, F3), and 50% replaced by KCl and $CaCl_2$ (1.25% NaCl, 0.625% KCl and 0.625% $CaCl_2$, F4). Changes in the composition of volatile compounds were studied during processing (0, 7, and 19 days) and storage (30, 60, and 90 days). Neither reduction nor replacement of NaCl by KCl affected the volatile compounds produced during the manufacturing process, and both increased the volatile compounds from carbohydrates fermentation and amino acid degradation during storage. The addition of $CaCl_2$ improved the generation of hexanal and (E)-hept-2-enal and other volatiles from lipid oxidation during processing and storage. After 90 days of storage, the control sample showed an increase in the generation of volatile compounds from lipid oxidation.

Keywords: dry fermented sausages, salt replacers, volatile compounds, lipid oxidation.

1 Introduction

Dry fermented sausages usually contain a high sodium chloride content (4–6%) (Stahnke & Tjener, 2007). This ingredient shows a number of effects, most of them desirable, including salty taste, the reduction of water activity and hence the control of microbial spoilage, the myofibrillar protein solubilization, and the control of the biochemical and enzymatic reactions during ripening, which affects the final aroma (Lücke, 1998).

However, from a health point of view, excessive sodium intake from NaCl addition in formulation is not recommended. For this reason, several authors have focused on the reduction or partial replacement of salt by KCl and/or $CaCl_2$ in dry fermented sausages (Gimeno, Astiasarán & Bello, 1998, 2001; Flores, Nieto, Ferrer & Flores, 2005; Zanardi, Ghidini, Conter & Lanieri, 2010; Campagnol, Santos, Terra & Pollonio, 2012). Nevertheless, the reduction of NaCl content can affect both the sensory quality (Corral, Salvador & Flores, 2013) and the safety of the process during the beginning of fermentation, when the water activity of the product is still high. The replacement of NaCl, particularly above 40% NaCl by KCl reduces the salty taste, replacing it with a bitter and metallic taste (Armenteros, Aristoy, Barat & Toldrá, 2012; Santos, Campagnol, Morgano & Pollonio, 2014), whereas the use of $CaCl_2$ increases lipid oxidation and affects product taste and texture (Gimeno et al. 1998; Flores et al. 2005; Ripollés, Campagnol, Armenteros, Aristoy & Toldrá, 2011).

Moreover, salt reduction or its partial replacement affects the generation of aroma active compounds (Corral et al. 2013; Campagnol, Santos, Wagner, Terra & Pollonio, 2011). Aroma formation in dry fermented sausages is mainly the result of lipolysis and proteolysis (Toldrá, 1998). Lipolysis generates free fatty

acids (FFA), which when subjected to lipid oxidation, produce a large variety of volatile compounds, including aldehydes, methyl ketones, and alcohols (Ordóñez, Hierro, Bruna & de la Hoz, 1999). Conversely, proteolysis is the generation of peptides and free amino acids from the gradual enzymatic degradation of the major myofibrillar and sarcoplasmic proteins. Free amino acids will contribute to the flavor development through chemical reactions such as the Strecker degradation (Flores, Sanz, Spanier, Aristoy, & Toldrá, 1998) or those caused by microorganisms such as decarboxylation by microbial decarboxylase (Ordóñez et al. 1999).

Salt concentration influences the activity of several muscle and microbial enzymes involved in the aroma development of dry fermented sausages (Toldrá, Flores, Aristoy, Navarro & Flores, 1997). Although the role of salt as a regulator of biochemical and enzymatic reactions is well known, there is little information on the effect of reduction or replacement of NaCl by other chloride salts on the generation of volatile compounds in dry fermented sausages, especially during the shelf life.

Reducing sodium in Brazilian meat products is part of a national agreement involving governmental agencies, meat industrial segment, consumers´ perspective, and food supply chain and storage (National Health Surveillance Agency (ANVISA), 2012). To find technological solutions to preserve the original properties of reformulated meat products when compared with traditional formulations throughout the shelf life, national sodium reduction goals are necessary. Another important point is the study of sensory quality of meat products with reduced or partial replacement of NaCl. In a recent study, dos Santos, Campagnol, Cruz, Morgano, Wagner and Pollonio (2015a) observed a

low sensory acceptance of dry fermented sausages with reduced NaCl content. However, a potential consumer market has been observed for fermented sausages containing 50% NaCl and 50% KCl, or 50% NaCl, 25% KCl and 25% $CaCl_2$. Alth ough many studies have addressed the effect of reducing or partially replacing NaCl by other chloride salts, a detailed study on the production of volatile compounds during processing and shelf life of dry fermented sausages is scarce.Therefore, the aim of this study was to investigate the effect of reduction or replacement of 50% NaCl by KCl, $CaCl_2$, and a blend of KCl and $CaCl_2$ on the volatile compounds generated during processing and storage of dry fermented sausages, considering the relevance of these compounds as an index of future sensory acceptance.

2 Material and methods

2.1 Manufacturing and sampling of dry fermented sausages

Treatments with 50% reduction or replacement of NaCl by KCl, $CaCl_2$ and a blend of KCl and $CaCl_2$ (1:1) were prepared as follows: control (2.5% NaCl), 50% salt reduced (1.25% NaCl, F1), 50% replaced by KCl (1.25% NaCl and 1.25% KCl, F2), 50% replaced by $CaCl_2$ (1.25% NaCl and 1.25% $CaCl_2$, F3), and 25% replaced by KCl and $CaCl_2$ (1.25% NaCl, 0.625% KCl and 0.625% $CaCl_2$, F4). The manufacturing process was as described by dos Santos et al. (2015). The raw materials used in this experiment were purchased from an industrial supplier under federal inspection (JBS, Brazil). Briefly, the pork meat (650 g/kg; moisture: 75.98 ± 0.25%, protein: 19.00 ± 0.52%, lipids: 3.43 ± 0.10%, and ash: 0,97 ± 0.81%), beef (200 g/kg; moisture: 76.12 ± 0.42%, protein: 19.57 ± 0.22%,

lipids: 3.20 ± 0.52%, and ash: 1.01 ± 0.41%), and pork back fat (150 g/kg; moisture: 16.78 ± 1.27%, protein: 6.54 ± 0.55%, lipids: 75.39 ± 0.25%, and ash: 0.35% ± 0.75%) were mixed with the respective amount of NaCl and other ingredients for each treatment. The following ingredients were added to the meat mixture in each treatment: glucose (5 g/kg), sucrose (5 g/kg), sodium nitrate (0.15 g/kg), sodium nitrite (0.15 g/kg), sodium ascorbate (0.25 g/kg), white powdered pepper (2 g/kg), garlic powder (3 g/kg), nutmeg powder (0.02 g/kg) and Starter culture (0.25 g/kg; SPX Floracarn, Chr Hansen, São Paulo, Brazil). After complete homogenization, the meat mixture was stuffed into cellulose casings of 50-mm diameter, and the sausages were subjected to a bath of 20% potassium sorbate solution. For each treatment, 65 sausages were produced of approximately 300 g each. The sausages were stored in a chamber for 19 days with the following programming of temperature and relative humidity: first day, 25 ºC/ 95%; second day 24 ºC/ 93%; third day, 23 ºC/ 90%; fourth day, 22 ºC/ 85%; fifth day 21 ºC/80%; sixth day 20 ºC/ 75%, and seventh day until the end of the ripening period (19 days), 18 ºC/ 75%. Air velocity was maintained at less than 0.5 m / s. After manufacturing, the casings were removed, and the samples were vacuum-packed and stored at 25 ºC for 90 days.

2.2 Analysis of volatile compounds

The volatile compounds were determined at the beginning of manufacture (0 days), at the end of fermentation (7 days), at the end of processing (19 days), and after 30, 60, and 90 days of refrigerated storage. For each period, three pieces of each treatment were collected and immediately frozen at -80 °C until the time of analysis. The extraction of headspace volatile compounds was

performed using a solid-phase micro extraction (SPME) as described by Wagner and Franco (2012), with a sorbent phase-Carboxen PDMS fiber (75 µm, 10 mm, Supelco, Bellefonte, PA, USA). Five grams of the sample were homogenized in a processor (Retsch, USA) at 3000 rpm for 1 min and placed into a 20 mL vial sealed with a PTFE faced silicone septum. The vial with the sample remained in a water bath at 50 °C for 15 min to reach equilibrium. Shortly thereafter, the fiber was exposed to the headspace for 45 min at 50 °C.

The identification and quantification of volatile compounds was performed on a gas chromatograph coupled to a mass spectrometer (GC/MS; SHIMADZU, QP-2010 Plus, Tokyo, Japan). The compounds adsorbed by the fiber were desorbed at a temperature of 250 °C in a split / splitless injection port in the splitless mode for 1 min. The separation was performed using a CP-WAX 52 CB column (Chrompack, USA) with the dimensions of 60 m x 0.25 mm i.d. x 0.25 µm film thickness. Helium was used as carrier gas under constant flow of 1.6 ml min^{-1}. The column temperature was set to 35 °C and maintained for 5 min, then at a gradient of 2 °C min^{-1} to reach 80 °C, followed by 4 °C min^{-1} to 210 °C, held for 5 min. A homologous series of alkanes (C6-C24) was analyzed under the same chromatographic conditions to calculate the linear retention indices (LRI) of the volatile compounds. The mass spectra were collected in the electron ionization mode (70 eV), at 35-350 m/z, and voltage detector of 1.2 kV. Compounds were identified by comparison of experimental mass spectra with those provided by the library of the National Institute of Standards and Technology (NIST 05). Additionally, experimental LRI were compared with the literature, and the order of elution of the compounds was observed and further compared with those found in the literature (Acree & Heinrich, 2014; El-Sayed, 2014). When available as a

pure substance, the mass spectra and LRI of the analytes and standards were compared and considered as positively identified. The standard compounds butanoic acid, pentanoic acid, butanal, hexan-1-ol, butan-2-one, octanoic acid, alpha pinene, beta-pinene, limonene were purchased from Sigma-Aldrich, USA) and ethanol, 3-methyl-1-butanol, ethyl acetate, and acetic acid from (Vetec, Brazil). The arbitrary peak areas were obtained from GC/MS total ion current chromatogram (TIC) operating in full scan mode.

2.3 Statistical Analysis

The data of the volatile compounds obtained at the end of the manufacturing process (19) were assessed by analysis of variance (one-way ANOVA), and means were compared using Tukey's test, with significance level of 5% ($p \leq 0\ 05$) using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). To assess the contribution of the major volatile compounds during manufacturing (0, 7, and 19 days) and storage (30, 60 and 90 days), the data were evaluated by multivariate principal component analysis (PCA) using the statistical program Pirouette 3:11 (2003).

3 Results and discussion

3.1 Volatile compounds in the final manufacturing

Sixty volatile compounds were identified at the end of the manufacturing process of the dry fermented sausages (day 19), with pH values ranging from 4.51 (50% $CaCl_2$) to 4.85 (F2- 50% KCl) and water activity of 0.873 for F2 (50%

KCl) and 0.915 for F1 (50% NaCl). Aliphatic hydrocarbons (branched or unbranched) were excluded from Table 1 because they do not have a significant aroma or because they were determined to be contaminants (Stahnke, 1995).

The mixture comprised nine aldehydes, eight alcohols, five ketones, seven acids, two esters, nine sulfides, one phenol, one ether, one furan, and 17 terpenes. The volatile compounds were grouped according to their likely origin (Ordóñez et al. 1999). Ten compounds were from carbohydrate fermentation, six from microbial metabolism, seven from lipid oxidation, thirteen from amino acid degradation, and twenty four from the condiments. Many of these volatile compounds have more than one origin or result from secondary reactions, such as acetic acid, which is primarily a product of the carbohydrates fermentation, but they also can be produced during the catabolism of amino acids and lipids (Viallon et al. 1996).

The volatile compounds from carbohydrate fermentation were the most abundant, representing anywhere from 43 to 61% of the total extracted area. Among these compounds, acetic acid was extracted with the highest total area (28-37%). Similar results were obtained by Corral et al. (2013) in low-sodium dry fermented sausages. The volatile compounds from the condiments represent the second largest total area extracted (32-45%), followed by the compounds from lipid oxidation (1-7%), microbial catabolism (2-3%), and amino acid catabolism (2-3%).

The reduction or replacement of NaCl by KCl and / or $CaCl_2$ significantly altered the volatile compounds from carbohydrates fermentation at the end of the manufacturing process (19). The acetic acid content increased in treatments F1 (50% NaCl) and F2 (50% NaCl and 50% KCl) when compared with control. It

should be noted that this compound has great impact on the flavor of the dry fermented sausages, contributing to the ripened aroma (Marco, Navarro & Flores, 2007; Olivares, Navarro & Flores, 2011) and may be produced by both homofermentative lactic acid bacteria and staphylococci, as well as by fatty acid oxidation and alanine catabolism (Montel, Seronine, Talon & Hebraud, 1995). In addition, F1 also had a higher concentration of 2-butanone than the control. The 2-ketones such as butan-2-one are commonly related to the aroma of butter and spicy notes in cured meat products (Flores, Grimm, Toldrá & Spanier, 1997). In contrast, the treatment containing $CaCl_2$ (F3 and F4) had a higher acetaldehyde concentration as well as higher concentrations of 3-methylbut-2-en-1-ol when compared with the control. Higher concentrations of acetic acid, 2-butanone, acetaldehyde, and 3-methylbut-2-en-1-ol were also found by Campagnol et al. (2011) in dry fermented sausages with replacement of NaCl by KCl and addition of yeast extract. Other authors have also reported that the reduction or replacement of NaCl by KCl or $CaCl_2$ affect the volatile compounds from carbohydrates fermentation (Flores et al. 2005; Corral et al. 2013).

Of the 13 compounds derived from amino acid catabolism, four were affected by the reduction or replacement of NaCl by KCl and / or $CaCl_2$. The treatments F1, F2, and F3 with 50% NaCl, 50% KCl, and 50% of $CaCl_2$, respectively, had a higher concentration of the compounds 3-methylbutanal and 2-methylbutanal than the control. These compounds are generated from the degradation of leucine and isoleucine, respectively and contribute to the overall flavor of fermented meat products by providing nutty, cheese, and salty notes (Andrés, Cava & Ruiz, 2002). Additionally, the concentration of 2-phenylethanol from the phenylalanine degradation was higher in F2 and F3 treatments when

compared with the control. The 2-phenylethanol gives winery notes to dry fermented sausages (Marco et al. 2007). Furthermore, the treatment F2 also had a higher concentration of 3-methylbutanoic acid. The increase in concentration of compounds from amino acid degradation in low-sodium dry fermented sausages has also been reported by other authors (Campagnol et al. 2011; Flores et. al. 2005; Stahnke, 1995). The changes in the profile of the volatile compounds from carbohydrate fermentation and amino acid catabolism observed in the present study may be due to changes in pH and water activity that results from the reduction or replacement of NaCl, which likely impact the metabolism of the starter cultures (Olivares, Navarro, Salvador & Flores, 2010).

The compounds from microbial catabolism contribute significantly to the development of characteristic aroma of dry fermented sausages, due to the production of compounds responsible for the fruity aroma, as the methyl pentanoate ester found in the study (Stahnke, 1995). In this study, the reduction or replacement of NaCl by KCl and / or $CaCl_2$ did not significantly influence the compounds from microbial catabolism at the end of the manufacturing process (19). Butanoic acid was the most abundant compound, which gives cheese notes, having great impact on the flavor of dry fermented sausages (Marco et al. 2007; Corral et al. 2013; Olivares et al. 2011). It is worth mentioning that the final aroma of fermented meat products is due to the combination and correct proportion of each type of volatile compound. Marco et al. (2007) have reported that compounds such as butanoic acid, 3-methyl butanoic acid, hexanal, and acetic acid are essential to the overall aroma of cured products and together with other esters, aldehydes, and acids contribute positively to the quality of the flavor of dry fermented sausages.

Lipid oxidation generates compounds of different chemical classes such as aldehydes, methyl ketones, and alcohols (Talon, 2004). Due to their low odor threshold, these compounds are of great importance to the aroma of dry fermented sausages (Ordóñez et al. 1999). The replacement of NaCl by $CaCl_2$ significantly affected the generation of volatile compounds from lipid oxidation at the end of the manufacturing process (19). The treatments with $CaCl_2$ (F3 and F4) resulted in a higher hexanal concentration. This compound is one of the main markers of lipid oxidation in dry fermented sausages and at high concentrations confers a rancid flavor (Brunton, Cronin, Monahan & Durcan, 2000). Among all treatments, F4 presented the highest hexanal content ($P < 0.05$). In addition to hexanal, the treatments F3 and F4 also produced a higher concentration of hexan-1-ol, whereas F4 also had a higher concentration of butanal, pentanal, (E)-hept-2-enal, and 2-ethylhexan-1-ol. The highest concentration of compounds derived from lipid oxidation in F3 and F4 may be associated with a higher ionic strength of the $CaCl_2$ when compared with NaCl and KCl (Rhee, Smith & Terrel, 1983; Hernandéz, Park & Rhee, 2002). The effect of ionic strength on lipid oxidation was demonstrated by Hernández et al. (2002), who studied a mixture of NaCl (0.625, 1.25, and 2.5%) and KCl (0.8, 1.6, 3.2%) in ground pork, with the three levels of NaCl and KCl corresponding to three ionic strengths, 0.175, 0.35, and 0.70, respectively. The authors reported that the pork meat treated with a mixture of higher ionic strength (0.70) had higher TBARS values.

The addition of condiments is very important in the manufacture of dry fermented sausages, mainly due to the development of characteristic flavor. Significant differences in the volatile compounds from condiments were observed between the control and the treatments. However, it was not possible to establish

whether these differences were caused by the reduction or replacement of NaCl by KCl and / or $CaCl_2$. These differences may be due to the non-uniform distribution of condiments in the samples subjected to SPME- GC / MS as observed by Coloretti et al. (2014).

Table 1- Volatile compounds (average area x 10^6) of dry fermented sausages at the end of the manufacturing process.

Identified compound	LRI	I[3]	Final product (19 days)				
			Control	F1	F2	F3	F4
Ethyl acetate	639	A	2.20±0.24^{a1}	1.72±0.26^{a}	0.51±0.47^{b}	1.95±0.11^{a}	0.98±0.06^{b}
Acetaldehyde	750	B	1.39±0.38^{d}	1.91±0.07^{bc}	1.76±0.13^{cd}	2.34±0.07^{ab}	2.43±0.05^{a}
Propan-2-one	829	B	2.30±0.14^{a}	3.07±0.19^{a}	2.51±0.87^{a}	0.77±0.02^{b}	2.89±0.37^{a}
Butan-2-one	913	A	2.43±0.70^{b}	6.04±0.31^{a}	3.46±0.39^{b}	3.55±0.25^{b}	2.55±0.40^{b}
Ethanol	944	A	1.28±0.02^{ab}	0.96±0.09^{b}	0.82±0.32^{b}	1.46±0.14^{a}	0.92±0.16^{b}
Butane-2,3-dione	995	B	5.47±0.61^{a}	4.26±0.62^{ab}	4.36±0.59^{ab}	4.93±0.47^{ab}	3.15±1.03^{b}
3-Hydroxybutan-2-one	1301	B	53.86 ±4.70	80.70±1.18	85.53±4.06	70.89±1.95	53.32±4.20
3-Methylbut-2-en-1-ol	1338	B	0.19±0.05^{c}	0.46±0.02^{ab}	0.42±0.01^{b}	0.41±0.03^{b}	0.53±0.02^{a}
Acetic acid	1462	A	125.76±7.30^{c}	157.17±4.42^{ab}	174.34±10.29^{a}	138.92±3.62^{bc}	138.95±9.06^{bc}
Butanoic acid	1643	A	9.53 ±2.76	8.80±4.40	12.78±0.37	9.26±0.30	9.16±0.41
Total Carbohydrate Catabolism			**204.41**	**265.09**	**286.49**	**234.48**	**214.88**
Methanethiol	703	B	2.55±1.24	3.40±0.74	3.61±0.51	3.71±1.05	3.11±0.28
2-Methylbutanal	920	B	0.31±0.13^{c}	0.73±0.06^{a}	0.64±0.04^{ab}	0.59±0.04^{ab}	0.46±009^{bc}
3-Methylbutanal	924	B	0.87±0.76^{b}	2.01±0.09^{a}	1.91±0.20^{a}	2.27±0.14^{a}	0.78±0.19^{b}
Dimethyl disulfide	1075	B	0.73±0.03	0.56±0.13	0.67±0.06	0.64±0.09	0.32±0.02
2-Methylbut-2-enal	1107	B	0.21±0.18	ND[2]	0.19±0.04	0.29±0.07	2.89±0.37

2-Methyl-1-butanol	1218	B	0.03±0.00	ND	ND	0.14±0.02	0.05±0.01
3-Methyl-1-butanol	1222	A	0.25±0.17	0.48±0.24	0.58±0.21	0.53±0.01	0.23±0.14
Benzaldehyde	1550	A	0.23±0.03	0.31±0.04	0.31±0.03	0.37±0.12	0.44±0.14
3-Methylbutanoic acid	1684	B	0.65±0.58^{b}	1.64±0.06^{ab}	2.10±0.06^{a}	1.08±0.12^{ab}	0.73±0.03^{b}
1-Phenylethanone	1688	B	0.86±0.50	1.30±0.15	1.55±0.27	1.34±0.08	1.54±0.14
Phenylmethanol	1912	B	0.21±0.07	0.25±0.03	ND	0.26±0.00	0.26±0.03
2-Phenylethanol	1940	B	0.55±0.03^{b}	1.17±0.19^{ab}	1.55±0.10^{a}	1.51±0.16^{a}	0.90±0.07^{ab}
Phenol	2034	B	0.26±0.11	0.33±0.03	0.37±0.04	0.37±0.04	0.38±0.02
Total Amino acid catabolism			**8.09**	**12.51**	**13.85**	**13.47**	**12.47**
Methyl pentanoate	1106	B	0.10±0.05	0.04±0.03	0.12±0.02	0.06±0.01	0.71±0.05
Propanoic acid	1552	B	0.87±0.05	1.00±0.04	1.15±0.28	1.02±0.13	0.91±0.08
Total Microbial Catabolism			**1.46**	**1.63**	**1.81**	**1.40**	**1.90**
Butanal	890	A	ND	ND	ND	ND	0.61±0.04
2-Methylfuran	882	B	3.94±0.22^{b}	5.23±0.21^{a}	4.35±0.19^{b}	4.45±0.24^{b}	3.82±0.43^{b}
Pentanal	990	B	1.71±0.56^{b}	1.61±0.18^{b}	1.09±0.24^{b}	1.67±0.21^{b}	2.43±0.05^{a}
2-Methylpropanoic acid	997	B	0.49±0.18	0.59±0.19	0.54±0.07	0.32±0.02	0.28±0.04
Hexanal	1089	B	1.03±0.15^{c}	ND	0.50±0.13^{c}	4.26±1.27^{b}	20.12±8.07^{a}
(E)-hept-2-enal	1331	B	ND	ND	ND	ND	0.81±0.48
Hexan-1-ol	1365	A	0.44±0.22^{c}	0.35±0.02^{c}	0.44±0.05^{c}	1.84±0.15^{b}	5.68±0.86^{a}
2-Ethylhexan-1-ol	1499	B	ND	0.27±0.03^{b}	ND	0.36±0.03^{ab}	0.45±0.08^{a}
Pentanoic acid	1656	A	0.32±0.01	0.30±0.02	0.27±0.14	0.28±0.01	0.27±0.05

Octanoic acid	2070	A	0.31±0.15	0.47±0.09	0.53±0.05	0.51±0.04	0.53±0.03
Total Lipid Oxidation			**7.75**	**8.23**	**7.18**	**13.37**	**34.72**
Allyl mercaptan	822	B	0.49±0.06	0.52±0.26	0.20±0.04	ND	0.23±0.04
2-Propen-1-thiol	897	B	58.70±18.00^{ab}	28.51±5.06^{bc}	10.97±15.82^{c}	64.22±2.94^{a}	55.30±7.76^{ab}
Allyl methyl sulfide	957	B	42.01±4.89^{a}	37.95±1.02^{ab}	39.52±0.59^{ab}	33.40±1.18^{bc}	27.48±2.34^{c}
Alpha-pinene	1016	A	2.60±0.13^{ab}	2.93±0.12^{a}	2.44±0.13^{b}	2.69±0.11^{ab}	2.79±0.20^{ab}
2-Methylthiophene	1095	B	17.07±1.77^{a}	5.86±0.55^{b}	4.66±0.37^{b}	4.81±0.41^{b}	4.97±0.32^{b}
Para-pinene	1097	A	ND	7.00±0.41^{b}	6.23±0.80^{b}	8.85±0.81^{b}	43.30±4.47^{a}
Sabinene	1110	B	0.39±0.09^{c}	0.54±0.07^{bc}	0.55±0.04^{bc}	0.60±0.04^{b}	0.83±0.07^{a}
Carene	1131	B	11.18±1.68^{b}	13.82±0.51^{a}	12.19±0.97^{ab}	11.97±0.24^{ab}	14.02±0.74^{a}
Diallyl sulfide	1137	B	16.14±13.54	24.15±1.04	21.03±0.50	19.27±0.23	19.09±1.50
Myrcene	1148	B	0.79±0.25^{b}	1.31±0.09^{a}	1.22±0.17^{a}	1.06±0.08^{ab}	1.21±0.08^{a}
Alpha-terpinene	1157	B	1.60±0.29^{b}	2.42±0.15^{a}	2.47±0.13^{a}	1.65±0.06^{b}	0.99±0.06^{c}
Limonene	1175	A	7.90±1.11^{b}	9.55±1.00^{ab}	9.48±0.77^{ab}	8.50±0.17^{ab}	10.59±0.99^{a}
Beta-phellandrene	1177	B	ND	ND	ND	1.21±0.02	2.19±0.08
Sabinene	1184	B	1.65±0.42	ND	1.58±1.37	1.43±0.24	2.70±0.45
1,8-cineole	1193	B	0.02±0.04	0.08±0.00	0.06±0.04	0.02±0.00	0.07±0.01
Para-cymene	1245	B	10.25±1.71	11.59±0.37	11.84±0.87	10.26±0.81	10.35±1.21
Alpha-terpinolene	1258	B	0.14±0.24	ND	0.20±0.03	0.54±0.04	0.21±0.06
Methyl allyl dissulfide	1267	B	4.70±3.66^{b}	7.20±0.99^{a}	8.31±0.49^{a}	7.34±0.19^{a}	6.48±0.24^{a}
Diallyldisulfide	1484	B	13.76±0.85	10.95±1.79	12.60±0.99	15.69±1.16	19.77±1.23

Linalool	1561	B	0.51±0.37	0.76±0.12	0.88±0.14	0.63±0.07	0.85±0.04
Isocaryophyllene	1573	B	0.24±0.14	0.37±0.08	0.49±0.18	0.25±0.09	0.55±0.15
Caryophyllene	1582	B	0.88±0.01	0.13±0.11	0.10±0.09	0.27±0.11	0.33±0.17
Alpha-terpineol	1621	B	0.64±0.39	0.31±0.07	0.38±0.06	0.30±0.01	0.36±0.04
Methyl eugenol	2046	B	0.24±0.17	0.18±0.05	0.24±0.06	0.18±0.02	0.24±0.02
Total Spices			**192.53**	**166.15**	**147.66**	**195.14**	**224.90**

[1] Mean ± standard deviation

[2] ND- Not detect

LRI- Experimental Linear Retention Index (DB-Wax; J&W Scientific, Folsom, California, USA).

I[3] The reliability of identification is indicated by the following symbols. A=mass spectrum and retention time agreed with standards. B=mass spectrum and retention index agreed with literature data.

Averages in the same row followed by the same letter are not significantly different using Tukey's test ($p > 0.05$). Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl. 25% KCl and 25% $CaCl_2$.

3.2 Volatile compounds generated during processing and storage

The volatile composition of the samples were analyzed by Principal Component Analysis (PCA), which was used to visually explore the impact of reducing or replacing 50% NaCl by KCl and / or $CaCl_2$ in the generation of volatiles during processing and storage of the dry fermented sausages. To perform the PCA, terpene compounds and the hydrocarbons were excluded, in addition to those with equivalent areas and did not differ significantly between treatments by the Tukey's test ($p>0.05$).

Figure 1 shows the PCA analysis of the volatile compounds formed during manufacturing process (at zero, seven, and 19 days). Two principal components, PC1 and PC2 explained 62.8% of the total variance (49.9% for PC1, and 12.9% for PC2). The first principal component (PC1) discriminated treatments according to the processing time. Samples analyzed on day 0 appeared in the negative part of PC1 and were clearly separated from the dry fermented sausages analyzed at the end of fermentation (seven days) and maturation (19), which appeared in the positive part of PC1. When analyzing each treatment, it was observed that the maturation stage modified the volatile compounds formed during fermentation, with the dry fermented sausages at day 19 in the most positive part of PC1 when compared with the samples at day 7.

The second principal component (PC2) allowed the discrimination of the treatments between the processing times. On day 0, the control and the treatment F2 appeared in the positive part, whereas the treatments F1, F3, and F4 were located in the negative part of PC2. Both the control and the formulation F2 were characterized by the compounds allyl methyl sulphide, propan-2-one, ethanol,

and etoxyethane, and the compounds carbon disulphide and dimethyl disulphide characterized the formulations F1, F3, and F4. No discrimination was observed between treatments at day 7, thus indicating that the reduction or replacement of 50% NaCl by KCl and / or $CaCl_2$ did not affect the volatile compounds formed during fermentation. In contrast, at the end of maturation (day 19), the control and the treatments F1 and F2 appeared in the positive part, whereas F3 and F4 appeared in the negative part of PC2. In short, the principal component analysis showed that the reduction or replacement of 50% NaCl by KCl did not cause significant changes in the volatile compounds produced during the manufacturing process of the dry fermented sausages. However, the addition of $CaCl_2$ at levels of 25 and 50% replacement increased the volatile compounds from lipid oxidation.

The generation of volatile compounds increased after 30 days of storage, and new compounds from lipid oxidation, amino acid degradation, and microbial metabolism were observed. The compounds 1-propanol, 2-butoxyethanol, 2-butenal, (E)-hept-2-enal, 2-methylpropanoic acid, and 2-butylfuran derivatives from lipid oxidation were generated. These compounds are important to the flavor of fermented meat products and their beneficial or detrimental effect to the typical flavor of dry fermented sausages will depend on the amount of each compound. Among the derivatives from lipid oxidation, aldehydes are the main compounds that contribute to the flavor quality of cured meat products (Ordoñéz et al. 1999, Lorenzo & Carballo, 2015); for example, the (E)-hept-2-enal can contribute to rancid and dirty odor (Meynier, Novelli, Chizzolini, Zanardi & Gandemer, 1999), whereas the 2-methylpropanoic acid is correlated with increased rancid flavor and cheese-like notes (Stahnke, 1995). The compounds from amino acids

degradation, ethylbenzene, 2-methylpropanal, 3-(methylthio)-1-propanol, and 3-methylbut-2-enyl acetate, and the compounds from bacterial metabolism by lipid beta-oxidation, pentan-3-one and pentane-2,3-dione, were also found for the first time in the dry fermented sausages after 30 days of storage.

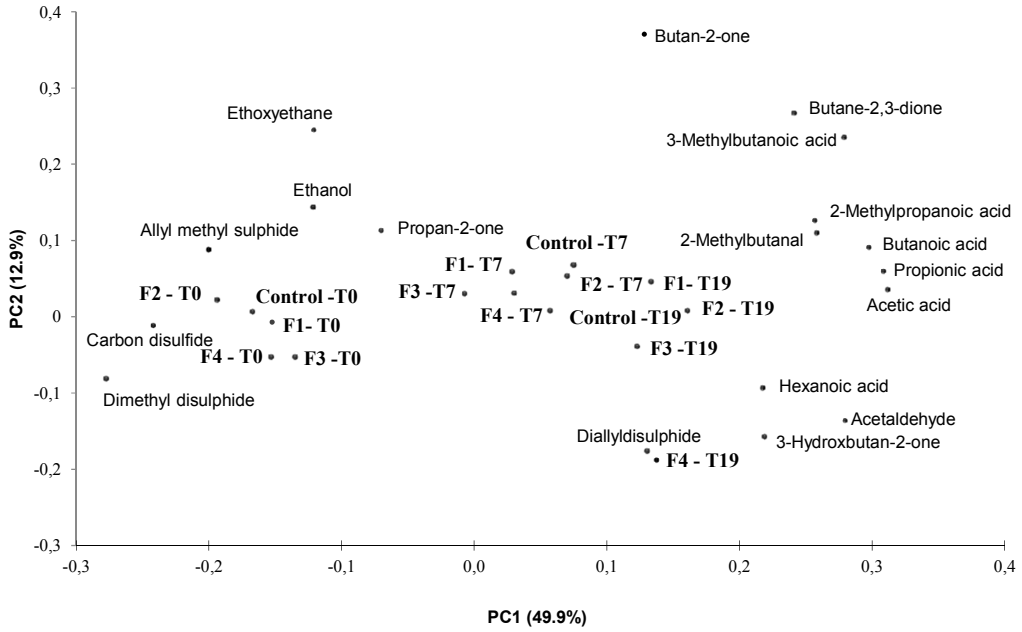

Figure 1. Loadings of the first two main components (PC1-PC2) of the volatile compounds for the dry fermented sausages with different salt contents (Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl. 25% KCl and 25% $CaCl_2$) at three ripening times (T0, T7 and T19).

Figure 2 shows the PCA analysis of the volatile compounds formed after 30 days of storage. Two principal components, PC1 and PC2 explained 76.59% of the total variance (46.5% for PC1, and 30 9% for PC2). The first two principal components showed that there was a separation of treatments into three groups. The control and the treatment F3 (50% $CaCl_2$) appeared in the negative part of PC1 and PC2, the treatments F1 (50% NaCl) and F2 (50% KCl) appeared in both

the negative part of PC1 and the positive part of PC2, and the treatment F4 (25% KCl and 25% $CaCl_2$) appeared in both the positive part of PC1 and the negative part of PC2. The control and the treatment F3 have been characterized by the compound 2-butanone from carbohydrate fermentation. Treatments F1 and F2 were characterized by compounds from carbohydrate fermentation (acetic acid, and butanoic acid), amino acid degradation (3-(methylthio)-1-propanol), lipid oxidation (2-methylpropanoic acid), condiments (3-methylsulfanylprop-1-ene), and unknown origin (ethoxyethane). The presence of 2-methylpropanoic acid in these treatments can contribute negatively to the flavor of the dry fermented sausages, as it provides rancid and cheese flavors (Stahnke, 1995). The treatment F4 was mainly characterized by compounds from lipid oxidation such as (E)-hept-2-enal (rancid and dirty odor), butanal (sweet and snacks odor), hexan-1-ol (green grass and plastic odor), and hexanal (fresh cut grass and rancid odor) (Marco et al. 2007). Furthermore, it was also characterized by compounds from microbial catabolism (pentane-2,3-dione and pente-3-one) and ethylbenzene, which is also derived from the amino acid degradation.

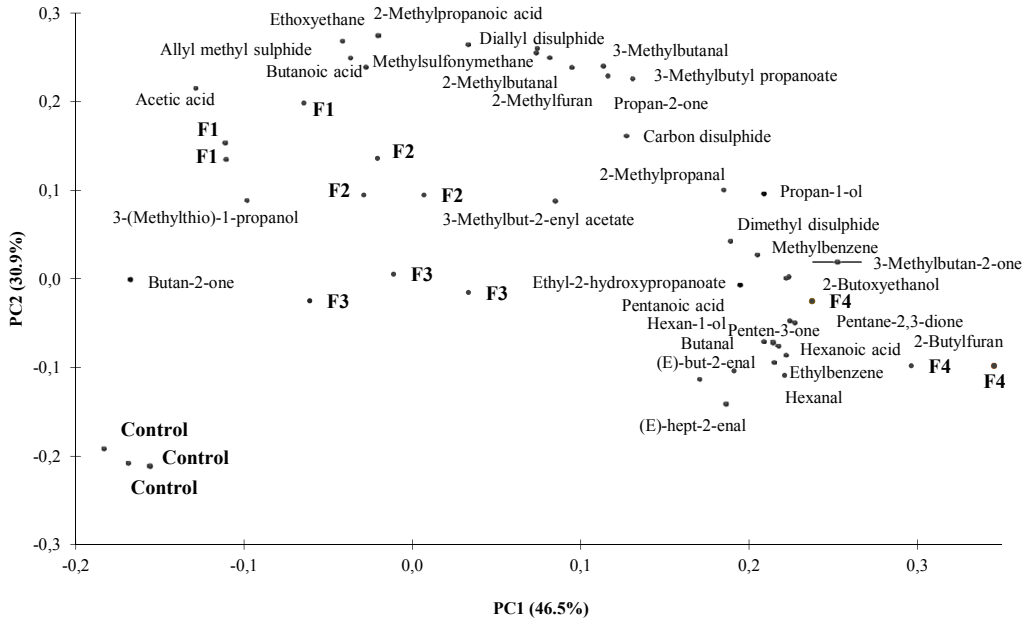

Figure 2. Loadings of the first two main components (PC1-PC2) of the volatile compounds for the dry fermented sausages with different salt contents (Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl, 25% KCl and 25% $CaCl_2$) at 30 days of storage.

After 60 days of storage, the first two principal components explained 72.9% of the total variance observed (Figure 3). The first principal component (PCI), which explained 56% of the total variance, discriminated the treatments into two groups. The control and the treatments F1 (50% NaCl) and F2 (50% NaCl and 50% KCl) appeared in the negative part of PCI, whereas the treatments F3 (50% NaCl and 50% CaCl2) and F4 (50% NaCl, 25% KCl and 25% CaCl2) appeared in the positive part of PCI. The control and the treatments F1 and F2 were mainly characterized by the volatile compounds 3-(methylthio)-1-propanol and 3-methylbutanal, which were generated from amino acid degradation and provide flavor of meat broth, rancid, savory and snacks (Marco et al. 2007) and rancid (Marco et al. 2007), respectively. In addition, compounds from the

carbohydrate fermentation (butan-2-one and butane-2,3-dione), microbial catabolism (butanoic acid), and lipid oxidation (2-methylpropanoic acid) also characterized the control and the treatments F1 and F2. Wu et al. (2014) also found higher contents of butan-2-one and butane-2,3-dione in bacons produced with 70% NaCl replaced by KCl. F3 and F4 were mainly characterized by several volatile compounds from lipid oxidation ((E)-but-2-enal, hexan-1-ol, 2-butoxyethanol, (E)-2-hepten-1-ol, 2-pentylfuran, (E)-oct-2-enal, hexanal, (E)-hept-2-enal, butanal, pentanoic acid, hexanoic acid, and heptanoic acid). In addition, F3 and F4 have also been characterized by the compound 2-pentylfuran, which was the only compound generated after 60 days of storage. This compound provides rancid odor (Marco et al. 2007) and has a great impact on the flavor of dry fermented sausages due to its low threshold. According to Andrés, Cava, Ventanas, Muriel and Ruiz (2007), 2-pentylfuran has been correlated with the NaCl content in fermented meat products, and the higher the NaCl concentration, the lower the concentration of this compound.

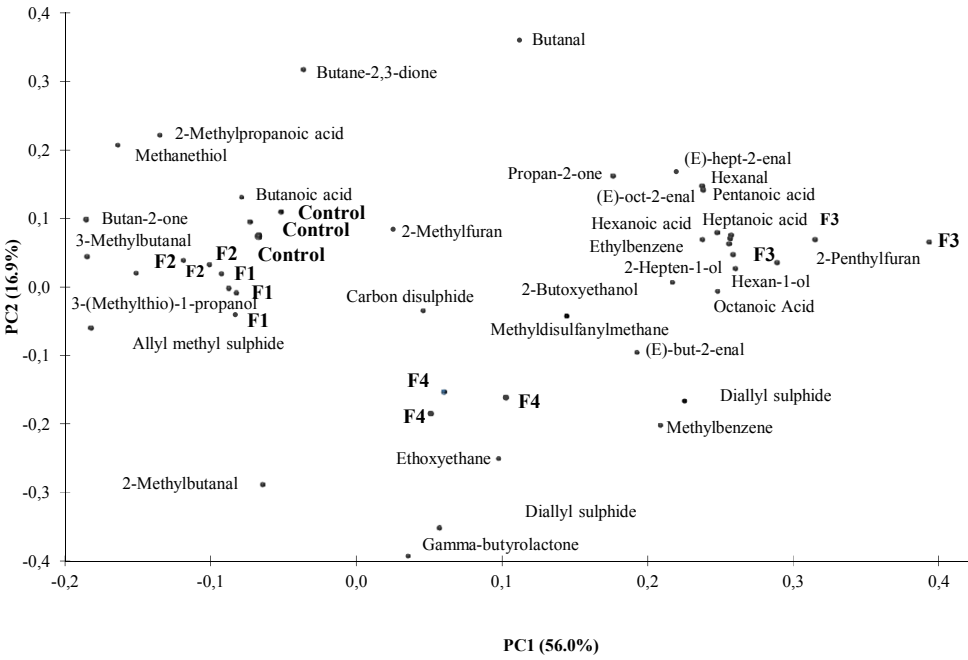

Figure 3. Loadings of the first two main components (PC1-PC2) of the volatile compounds for the dry fermented sausages with different salt contents (Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl. 25% KCl and 25% $CaCl_2$) at 60 days of storage.

The two first principal components that explained 60.2% of the total variance of the data discriminated the samples stored for 90 days into four groups (Figure 4). The samples located in the lower left quadrant (F1- 50% NaCl and F2 - 50% KCl) have been characterized mainly by the compounds derived from amino acids catabolism (2-methylpropanal, 2-methylbutanal, 3-methylbutanal, 2,6-dimethylpyrazine). This shows that a 50% NaCl reduction and 50% replacement of NaCl by KCl maintained important compounds for the characteristic flavor of dry fermented sausages (Ruiz, Ventanas, Cava, Andres & Garcia, 1999) even at the end of the storage time (90 days, Table 2). Moreover, the reductions were also characterized by compounds butyl acetate, ethanol, butan-2-one and 3-methylbutan-2-one, which were obtained from carbohydrate

fermentation. The treatment F4 (25% KCl, and 25 % $CaCl_2$), which appeared in the upper left quadrant was characterized mainly by compounds derived from the condiments. The control (lower right quadrant) and the treatment F3 (50% $CaCl_2$) (upper right quadrant) were mainly characterized by the compounds from lipid oxidation. Among them, (3E)-3-ethyl-2-methylhexa-1,3-diene, (2E,4E)-hepta-2,4-dienal, and octan-1-ol were formed only after 90 days of storage. The increase of volatile compounds from lipid oxidation in the control sausage at the end of storage highlights the pro-oxidant effect of NaCl when used in high concentrations, as reported by Coutron-Gambotti et al. (1999). Regarding the higher production of volatile compounds from lipid oxidation in the dry fermented sausages containing 50% $CaCl_2$, Zanardi et al. (2010) and Flores et al. (2005) observed similar behavior, finding an increase in lipid oxidation of sausages containing $CaCl_2$.

Therefore, the reduction of 50% NaCl (F1) or replacement of 50% NaCl by KCl (F2) decreased the volatiles from lipid oxidation, and increased the volatiles from carbohydrate fermentation and amino acid degradation, suggesting a lower lipid oxidation. The treatment containing $CaCl_2$ showed a progressive increase in lipid oxidation during storage; however, at the end of 90 days, the sample containing 50% $CaCl_2$ (F3) was characterized by a large number of compounds from lipid oxidation when compared with the treatment containing 25% $CaCl_2$ (F4). Furthermore, the increase in compounds from lipid oxidation in the dry fermented sausages containing only NaCl after 90 days of storage suggests that the sensory quality was decreased during storage.

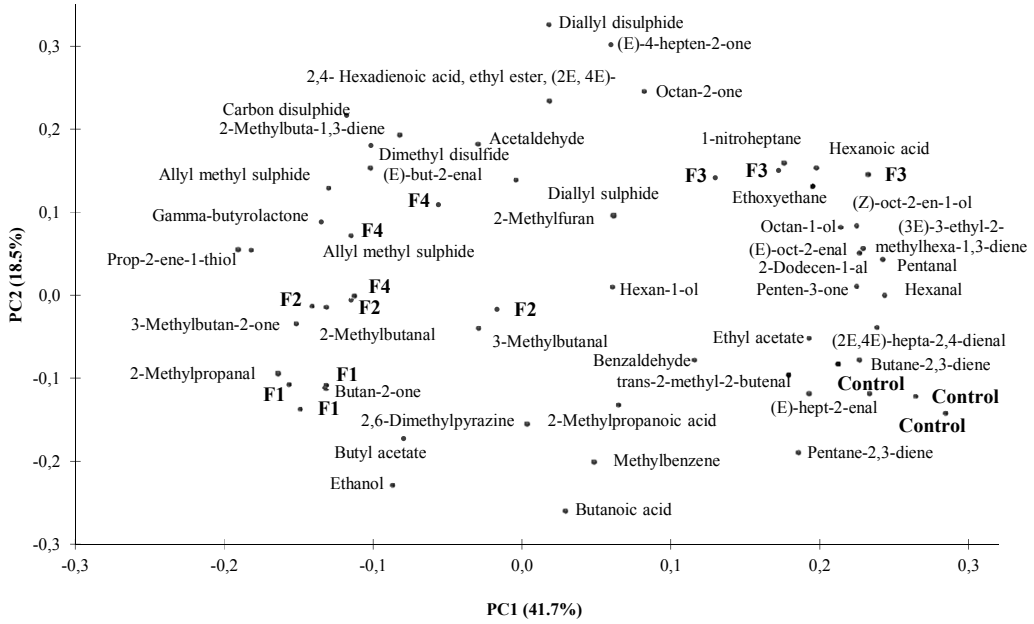

Figure 4. Loadings of the first two main components (PC1-PC2) of the volatile compounds for the dry fermented sausages with different salt contents (Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl. 25% KCl and 25% $CaCl_2$) at 90 days of storage.

Table 2- Volatile compounds (average area x 10^6) of dry fermented sausages at the end of the shelf life.

Identified compound	LRI	I[3]	End shelf life (90 days)				
			Control	F1	F2	F3	F4
Acetaldehyde	713	B	6.23±0.50^{ab1}	5.25±0.25^{b}	7.35±0.36^{ab}	7.09±0.45^{ab}	11.26±0.59^{a}
Ethyl acetate	899	A	0.07±0.02	0.10±0.03	0.10±0.01	0.12±0.04	0.16±0.05
Butan-2-one	944	A	8.03±0.14^{c}	14.70±1.12^{a}	7.10±0.59^{c}	5.84±0.56^{c}	12.24±0.33^{b}
3-Methylbutan-2-one	955	B	0.07±0.01^{b}	0.58±0.02^{ab}	1.14±0.07^{a}	ND[2]	0.58±0.01^{ab}
Ethanol	963	A	2.88±0.68^{ab}	3.97±0.68^{a}	2.80±0.20^{ab}	2.01±0.04^{b}	2.74±0.23^{b}
Butane-2,3-diene	993	B	14.21±1.04^{a}	8.45±0.39^{c}	10.72±0.60^{bc}	11.27±1.34^{b}	8.88±0.45^{c}
Butyl acetate	1075	B	0.45±0.02^{b}	1.59±0.26^{a}	0.75±0.04^{ab}	0.32±0.03^{b}	0.29±0.03^{b}
3-hidroxybutan-2-one	1301	B	27.49±5.91	22.60±12.52	30.58±5.80	8.15±5.00	18.15±9.85
2,3,5-trimethylpyrazine	1339	B	0.61±0.10	1.44±0.52	2.45±0.20	0.56±0.18	1.15±0.25
Acetic acid	1453	A	72.77±21.90	66.25±5.77	95.02±2.61	49.42±12.12	63.56±19.52
Butanoic acid	1643	A	16.58±0.89^{a}	14.89±0.73^{ab}	14.29±0.79^{b}	11.76±0.66^{c}	14.32±1.05^{ab}
Total Carbohydrate Catabolism			**149.39**	**139.82**	**172.30**	**96.54**	**133.33**
2-Methylbutanal	947	B	3.83±0.33^{c}	7.53±0.31^{b}	15.54±0.49^{a}	4.09±0.02^{c}	7.77±0.93^{b}
2-Phenylpropan-2-ol	1243	B	0.12±0.01	ND	0.13±0.02	0.14±0.02	0.19±0.02
2,6-dimethylpyrazine	1344	B	1.77±0.14^{ab}	0.68±0.05^{c}	2.30±0.38^{a}	ND	0.97±0.15^{bc}
Benzaldehyde	1543	A	4.99±0.44^{a}	4.73±0.46^{ab}	3.55±0.59^{b}	4.46±0.39^{ab}	4.54±0.61^{ab}
trans-3-methylbut-2-enal	1693	B	31.40±1.33^{a}	12.93±1.29^{ab}	8.87±0.80^{b}	16.29±4.30^{ab}	14.95±0.98^{ab}
2-Phenylethanol	>2000	B	ND	1.79±0.31	2.90±0.30	1.16±0.10	1.70±0.18
Phenol	>2000	B	0.73±0.05	0.53±0.03	0.98±0.04	0.82±0.06	0.83±0.03
Total Amino Acid Catabolism			**42.84**	**28.19**	**34.27**	**26.96**	**30.95**
Pentane-2,3-diene	741	B	12.10±0.90^{a}	1.29±0.93^{c}	3.52±1.03^{b}	2.09±0.62^{bc}	1.12±0.48^{c}
2-Methylpropanal	817	A	0.91±0.41^{b}	4.83±0.63^{a}	4.41±0.69^{ab}	0.97±0.55^{b}	3.11±1.04^{ab}
Penten-3-one	1027	B	3.74±0.71^{a}	ND	1.75±0.14^{bc}	2.67±0.56^{ab}	1.19±0.49^{c}
Octan-2-one	1280	B	0.01±0.00^{b}	0.12±0.02^{b}	0.03±0.02^{b}	1.27±0.09^{a}	0.60±0.02^{ab}
(Z)-Oct-2-en-1-ol	1622	B	0.27±0.06^{ab}	ND	0.14±0.01^{bc}	0.32±0.06^{a}	0.06±0.00^{c}
Total Microbial Catabolism			**17.03**	**6.24**	**9.85**	**7.32**	**6.08**

2-Methylbuta-1,3-diene	649	A	0.93±0.02^{b}	1.86±0.20^{ab}	2.16±0.12^{b}	1.77±0.10^{b}	3.56±0.09^{a}
Butanal	882	A	1.64±0.45	1.16±0.58	1.38±0.87	1.55±0.84	0.94±0.05
2-Methylfuran	858	B	8.58±0.28^{b}	8.93±0.89^{b}	12.94±0.99^{ab}	9.24±0.75^{b}	15.39±0.98^{a}
(E)-but-2-enal	883	B	4.47±1.16^{a}	0.70±0.60^{b}	3.65±0.16^{a}	5.20±1.34^{a}	2.88±0.67^{ab}
Allyl methyl sulphide	986	B	7.92±6.89^{c}	21.20±1.18^{ab}	4.17±0.25^{a}	3.04±0.26^{ab}	3.78±0.64^{a}
Pentanal	989	B	33.91±1.16^{a}	18.88±1.77^{c}	25.93±0.66^{b}	34.72±3.21^{a}	21.58±1.85^{bc}
Gamma-butyrolactone	1013	B	1.84±0.17^{b}	2.51±0.75^{b}	0.65±0.03^{a}	0.40±0.07^{ab}	0.44±0.07^{ab}
Hexanal	1087	B	189.74±11.10^{a}	184.39±10.60^{a}	138.32±7.85^{b}	107.71±19.41^{ab}	94.79±13.98^{b}
Penten-1-al	1135	B	14.17±8.20	13.81±11.50	18.62±0.68	34.39±7.84	24.68±10.56
(E)-4-Hepten-2-one	1244	B	ND	0.11±0.01^{b}	0.14±0.03^{b}	0.52±0.05^{a}	0.44±0.07^{a}
1-octen-3-one	1246	B	4.18±2.56	0.33±0.08	1.49±0.60	4.11±1.82	0.62±0.05
(E)-2-heptenal	1329	B	33.02±1.47^{a}	ND	6.61±1.40^{b}	8.02±1.52^{b}	7.33±2.11^{b}
Hexan-1-ol	1361	A	36.70±1.30^{ab}	40.74±1.89^{a}	33.24±1.93^{b}	40.61±0.84^{a}	35.55±2.66^{b}
(2E,4E)- hepta-2,4-dienal	1401	A	2.57±0.12^{a}	ND	0.78±0.03^{b}	1.17±0.09^{b}	0.43±0.16^{b}
2-Butoxyethanol	1409	A	0.85±0.07	0.99±0.08	1.46±0.09	1.36±0.11	1.31±0.30
(3E) -3-ethyl-2-methylhexa-1,3-diene	1420	B	3.25±0.09^{a}	0.42±0.37^{c}	1.01±0.18^{bc}	2.77±0.45^{a}	1.31±0.34^{b}
(E)-oct-2-enal	1437	B	6.70±0.08^{a}	0.55±0.04^{b}	1.98±0.08^{b}	5.19±1.20^{a}	1.66±0.99^{b}
Octan-1-ol	1565	A	2.46±0.25^{a}	1.22±0.10^{b}	1.13±0.21^{b}	2.53±0.28^{a}	1.64±0.59^{ab}
2-Methylpropanoic acid	1573	B	0.90±0.16^{a}	0.41±0.03^{ab}	0.65±0.06^{ab}	0.24±0.02^{b}	0.62±0.08^{ab}
2-Dodecen-1-al	1765	B	ND	ND	ND	0.22±0.03	ND
Hexanoic acid	1852	A	21.71±2.12^{ab}	9.80±2.66^{c}	14.78±1.18^{bc}	25.96±2.44^{a}	16.28±1.50^{bc}
Octanoic Acid	>2000	A	3.14±0.42	1.82±0.05	2.47±0.07	3.79±0.11	2.70±0.42
Decanoic acid	>2000	B	1.17±0.16	0.85±0.07	1.11±0.18	1.71±0.17	1.26±0.14
Total Lipid Oxidation			**383.00**	**320.13**	**303.03**	**309.13**	**262.05**
Alpha-pinene	1016	B	2.98±0.59	3.77±0.29	3.58±0.24	3.12±0.30	3.68±0.28
Dimethyl disulphide	1075	B	ND	0.28±0.02^{b}	0.62±0.04^{a}	0.40±0.07^{ab}	0.44±0.07^{ab}
Diallyl sulphide	1137	B	8.65±0.09^{b}	19.25±2.85^{a}	ND	20.76±4.93^{a}	21.90±3.85^{a}
Diallyl disulphide	1480	B	12.36±1.26^{bc}	7.54±1.59^{c}	16.32±0.66^{abc}	22.82±2.65^{a}	21.52±2.38^{ab}
Linalool	1557	B	1.57±0.15	2.34±0.64	1.63±0.10	1.68±0.03	1.75±0.11
Caryophyllene	1582	A	8.48±0.82	6.59±1.56	8.94±1.42	9.89±0.21	9.87±0.74
Isocaryophyllene	1597	A	5.78±0.32	5.50±0.81	8.33±1.70	9.04±2.10	7.28±0.63

Total Spices			**39.82**	**45.27**	**39.42**	**67.71**	**66.44**
Carbon disulphide	536	B	4.41±0.32^{c}	5.86±0.85^{bc}	8.28±0.57^{ab}	6.78±0.89^{bc}	10.08±1.02^{a}
Ethoxyethane	589	A	0.75±0.01^{ab}	0.68±0.02^{ab}	0.01±0.00^{b}	0.87±0.02^{a}	1.09±0.21^{a}
Methylbenzene	786	B	4.30±0.99^{a}	4.09±0.32^{a}	4.77±0.31^{a}	3.50±0.20^{ab}	2.36±0.08^{b}
1-Nitroheptane	1402	B	0.32±0.12^{ab}	0.02±0.00^{b}	0.16±0.02^{ab}	0.53±0.06^{a}	0.22±0.01^{ab}
2,4-Hexadienoic acid, ethyl ester, (2E,4E) -	1446	B	3.25±0.08^{a}	0.42±0.07^{c}	1.01±0.18^{bc}	2.77±0.45^{a}	1.31±0.03^{b}
Others and Unknown			**13.03**	**11.07**	**14.23**	**14.45**	**15.06**

[1] Mean ± standard deviation

[2] ND- Not detect

LRI- Experimental Linear Retention Index (DB-Wax; J&W Scientific. Folsom. California. USA).

I[3] The reliability of identification is indicated by the following symbols. A=mass spectrum and retention time agreed with standards. B=mass spectrum and retention index agreed with literature data.

Averages in the same row followed by the same letter are not significantly different using Tukey's test ($p > 0.05$). Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl. 25% KCl and 25% $CaCl_2$.

4 Conclusion

For the first time, a detailed study on the effect of partial replacement of NaCl on the generation of volatile compounds was carried out during the manufacturing process and shelf life of dry fermented sausages. Salt concentration of 2.5% presented a potent pro-oxidant effect as it led to the formation a greater number of volatile compounds from lipid oxidation at the end of storage. The $CaCl_2$ increased the generation of volatiles from lipid oxidation during processing and storage of dry fermented sausages, thus indicating that the replacement of 25 and 50% NaCl by $CaCl_2$ is not a viable alternative. The reduction of 50% NaCl and replacement of 50% NaCl by KCl did not cause significant changes in the generation of volatiles during processing of dry fermented sausages and attenuated lipid oxidation during storage.

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CAPÍTULO 4

LOW SODIUM BRAZILIAN SALAMIS: EFFECTS ON LIPID OXIDATION

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Abstract

Salamis that have reduce NaCl content or replacement that replace some of the salt with KCl, $CaCl_2$, or a blend of KCl and $CaCl_2$ were evaluated through TBARS analysis, fatty acid composition, and instrumental color for to examine their oxidative stability during processing and storage. We found that a 50% reduction in NaCl decreased the intensity of the reactions to lipid oxidation, while treatments with added $CaCl_2$ resulted in increased lipid oxidation during manufacture and storage. Fatty acid composition also changed owing to the presence of KCl and $CaCl_2$, showing a decrease in saturated, monounsaturated, and polyunsaturated fatty acids after 30 days of storage. Futher, a decreased intensity of L* and increased b* values were observed for salamis that had of 50% and 25% $CaCl_2$ added. These results suggest that using $CaCl_2$ as a substitute for NaCl increases the intensity of oxidative reactions in salamis.

***Keywords**:* Calcium Chloride, Lipid Oxidation, Salamis, Sodium Chloride, Unsaturated Fatty Acids.

Introduction

Lipolytic reactions result from the most important biochemical events occurring in the lipid fraction during the processing and storage of fermented meat products, directly influencing the overall quality of the final product (DEMEYER et al., 1974, RIPOLLÉS et al., 2011). These reactions are influenced by many factors, including the processing conditions, pH, presence of metals, and nature and contents of salt (QUINTANILLA et al., 1996). In particular, reactions involving lipid oxidation are important in the development of the aroma and flavor of salamis; however, they must be controlled to ensure that the excess and transformation of these compounds does not affect the desired characteristics or risks consumer health (WÓJCIAK and DOLATOWSKI, 2012).

Sodium chloride (NaCl) is one of the main ingredients used in salami processing, playing an important role in technological, microbiological, and sensory quality. This ingredient, however, is the main source of sodium, an element targeted for reduction throughout the production chain of processed foods, especially meat products (GOMES et al., 2011, HORITA et al., 2011, DOS SANTOS et al., 2014, DOS SANTOS et al., 2015).

Sodium chloride (NaCl) has been described by many authors as a pro-oxidant compound capable of influencing the development and intensity of lipid reactions in salamis. Its effect on lipid oxidation appears to be due either to the reactive action of chloride ions on lipids (LOVE and PEARSON, 1974) or to the solubilization of iron by chloride ions, stimulating lipid peroxidation (OSINCHACK et al., 1992). According to TOLDRÁ (1992), NaCl content can also interfere with the activity of endogenous enzymes, thus changing the intensity of lipolytic

reactions. Although the reduction of NaCl in fermented meat products has been widely studied to examine the biochemical changes affecting the quality of products; the effect of substitute salts, such as CaCl2 and KCl, still requires investigation. In particular, the effects of this reformulation on the attributes of lipid oxidation considering the molecular weight of each replacer or net weight are under-researched (ARMENTEROS et al., 2009, RIPOLLÉS et al., 2011, CORRAL et al., 2013, WU et al., 2015).

Further, strategies to reduce or replace the NaCl content in salamis in order to meet requirements for the overall reduction in consumption of NaCl in the diet (WORLD HEALTH ORGANIZATION (WHO), 2012) may affect the rate and degree of the development of lipid oxidation in salamis. Hence, this study contributes to the body of knowledge on this topic by evaluating the oxidative stability of reduced NaCl salamis during the curing process and storage of salamis that had their NaCl content reduced or replaced with KCl, $CaCl_2$, or a blend of KCl and $CaCl_2$.

Materials and Methods

Treatments and Processing of salamis

Treatments with 50% reduction or replacement of NaCl by KCl, $CaCl_2$, and a blend of KCl and CaCl2 (1:1) were prepared, as follows: control (2.5% NaCl), 50% salt reduced (1.25% NaCl, F1), 50% replaced by KCl (1.25% NaCl and 1.25% KCl, F2), 50% replaced by $CaCl_2$ (1.25% NaCl and 1.25% CaCl2, F3) and 50% replaced by KCl and $CaCl_2$ (1.25% NaCl, 0.625% KCl and 0.625 $CaCl_2$). Processing was carried out in the Laboratory of Meat and Derivatives at the

Faculty of Food Engineering, University of Campinas (São Paulo, Brazil). The manufacturing process was as described by DOS SANTOS et al. (2015). Salamis were produced with pork meat (650 g/kg), beef (200 g/kg), and pork back fat (150 g/kg) and were mixed with the correct amount of NaCl and other ingredients for each treatment. The following ingredients were added to the meat mixture in each treatment: glucose, sucrose, sodium nitrate, sodium nitrite, sodium ascorbate, white pepper, nutmeg, and starter culture (SPX Floracam, Chr. Hansen). After complete homogenization, the batter of each treatment was packaged and taken to the salami manufacturing chamber. For each treatment, 90 sausages were produced of approximately 300 g each. The parameters for temperature and relative humidity were as follows: 1st day (25°C/95%), 2nd day (24°C/93%), 3rd day (23°C/90%), 4th day (22°C/85%), 5th day (21°C/80%), 6th day (20°C/75%), and from 7th day until the end of maturation, 19 days total (18°C/75%). Air velocity was maintained below 5 m/s. At the end of the manufacturing process (19 days, or timepoint 0), salamis were vacuum-packed (Unipac/Univac B320-Minivac CU18, Selovac, São Paulo, SP, Brazil) and stored at 25 ± 1°for 90 days.

Lipid Oxidation and Fatty Acid Composition

Lipid oxidation and fatty acid composition were analyzed at the beginning of processing (day 0), at the end of fermentation (day 7), at the end of processing (day 19), and after 30, 60, and 90 days of storage at 25°C. For each timepoint, three pieces of each treatment were collected and immediately frozen at 80°C until analysis.

The lipid oxidation of salamis was measured by the amount of thiobarbituric acid-reactive substances (TBARS), as described by BRUNA et al. (2001), using trichloroacetic acid instead of perchloric acid as the solvent. The results were expressed in μg of malonaldehyde (MDA)/g for each sample.

Lipids were extracted using chloroform/methanol/water (1:2:0.6 V/V) as described by BLIGH and DYER (1959), and they underwent transesterification using the method of HARTMAN and LAGO (1973). Fatty acid methyl esters (FAMEs) were analyzed in a gas chromatograph equipped with a flame ionization detector (GC - FID), Varian Star 3400CX (CA, USA), by using an autosampler model 4200 (CA, USA). A 1μL injection of the sample was performed using a split type/splitless injector operating in split mode 1:50 at 250°C. Hydrogen was used as a carrier gas at a constant pressure of 30 psi and an initial flow rate of 0.8 ml/min. FAMEs were separated into capillary columns (SP - 2560 Supelco, USA; 100 m × 0.25 mm × 0.20 μm). The initial column heating temperature was 80°C, which was maintained for 30 s, followed by an increase of 15°C/min until 175°C was reached, followed by a second increase of 0.5°C/min until 190°C and a final increase of 8°C/min until 240°C. The column was then kept isothermal for 15 mins. The detector temperature was maintained at 240°C.

FAMEs were identified by comparing the retention times of the analytes with authentic standards FAME Mix-37, P / N 47885-U; methyl ester 11-trans-vaccenic acid, P / N 46905-U, mixed isomers of conjugated linoleic acid methyl esters, P / N 05632, mixed isomers of linoleic and linolenic acid methyl esters, P / N 47791 P and / N 47792; cis-7, 10, 13, 16, 19-docosapentaenoic acid methyl ester, P / N 47563-U, all produced by Supelco (PA, USA) and purchased from

Sigma-Aldrich. Quantification was carried out using the internal standard methyl tricosanoate (C23:0), and the quantitative method was validated by VISENTAINER (2012). The results were expressed in mg/g lipid.

Instrumental Color

Color was determined at the end of the curing process and storage using a spectrophotometer - colorimeter (model CM-5; Konica Minolta) with spectral reflectance included as a calibration mode, Standard Illuminant D65, and an observation angle of 10°, operating under the CIE system (L*a*b*). The values of L* (luminosity), a* (intensity of red), and b* (intensity of yellow) were determined. Whiteness value was calculated using the following formula (PARK, 1994):

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

where L*= lightness on a 0-100 scale from black to white, a*= scale of red (+) or green (-), and b*= scale of yellow (+) or blue (-).

Statistical Analysis

In each manufacture, three sample units (salamis) were taken per sampling day (n = 9). All analyses were performed in triplicate. The results reported in this study are the mean obtained from all the data recorded for each parameter analyzed. Data were evaluated by analysis of variance (one-way ANOVA) and Fisher's test ($P<0.05$) to identify the differences among salamis assessed during the curing process and over their shelf life. STATISTICA V.8 SOFTWARE was used for data analysis.

Results and Discussion

Effects of Salts on Lipid Oxidation

Lipid oxidation is one of the main phenomena responsible for the reduced shelf life and sensory quality of fermented meat products. Malondialdehyde (MDA) is a typical product of lipid oxidation, formed mainly by the degradation of polyunsaturated fatty acids (ERCOŞKUN and ÖZKAL, 2011). Table 1 shows the effect on lipolytic reactions (TBARS) of reducing or replacing NaCl with KCl and/or $CaCl_2$ during the manufacture and storage of salamis.

At the beginning of manufacture (0 days), the measured TBARS amounts were not significantly different among the treatments. On the 7th day of manufacture, only treatment F3 (50% NaCl and 50% $CaCl_2$) presented TBARS values higher than the control ($P<0.05$). At the end of the curing process (19/0d), the treatments with the addition of 50% (F3) and 25 % $CaCl_2$ (F4) showed significantly higher amounts of TBARS than did the control group. This behavior continued until 60 days of storage. At the final timepoint of 90 days, only salamis produced with a 50 % reduction of NaCl (F1) differed from the control group, with lower TBARS amounts.

There are many postulations as to how sodium chloride acts as a pro - oxidant. KANNER and ROSENTHAL (1992) argue that NaCl acts as a pro - oxidant by displacing the iron ions with sodium in the heme pigments of the muscle tissue, whereas others recognize the chloride ion acting upon the lipid as the source (ELLIS et al., 1970). The metal impurities, particularly iron, within salt are also thought to cause lipid oxidation (CHANG and WATTS 1950; DENISOV and EMANUEL 1960; SALIH 1986). According to RHEE et al. (1983), RHEE et

al. (1996), and HERNÁNDEZ et al. (2002), lipid oxidation is enhanced by increasing ionic strength. This finding can partly explain the greater lipid oxidation observed in treatments with $CaCl_2$, since the ionic strength of divalent salts is higher than that of monovalent salts. An increase in lipid oxidation with the addition of divalent salts was reported by ZANARDI et al. (2010), who replace NaCl with a blend of salts (KCl, $CaCl_2$, and $MgCl_2$) in Italian-type salamis. High TBARS levels were also found by FLORES et al. (2005) when using 0.5% $CaCl_2$ in salamis.

According to RHEE and ZIPRIN (2001), the addition of 0.5–2.5% NaCl in meat products is enough to provide a pro-oxidant effect, thus increasing oxidative reactions during the processing and storage stages of products. In their study, salamis produced with 50% reduction of NaCl (F1 - 1.25%) had lower TBARS values ($P \leq 0.05$) than the control group. This result clarifies the pro-oxidant effect of NaCl when used at a concentration of 2.5% (COUNTRON-GAMBOTI et al., 1999, HORITA et al., 2011).

In the evaluation of TBARS levels during the 90-day storage period, a progressive increase was observed in lipid oxidation across all treatments. This same behavior has been reported by other researchers studying the development of lipid oxidation in salamis (SANTOS et al., 2012, CORRAL et al., 2013, CORRAL et al., 2015). Increasing lipid oxidation can adversely affect the sensory quality of dry fermented sausages by generating compounds of degradation, such as n-alkenals and dienals (ANSORENA and ASTIASARÁN, 2004). Additionally, oxidation can affect the nutritional value of products by decomposing vitamins and unsaturated fatty acids (ANSORENA and ASTIASARÁN, 2004).

Table 1- Malondialdehyde content (µg MDA/g sample) during curing process and storage of dry fermented sausage with NaCl reduced or replaced by KCl and/or $CaCl_2$.

Treatments	Time (days)						
	0[a]	7	19/0 [b]	30	60	90	SEM*
Control	0.07^{aD}	0.126^{bC}	0·144^{bcBC}	0·170^{bB}	0·181^{cB}	0·337^{aA}	0.01
F1	0.09^{aD}	0.124^{bCD}	0·131^{cC}	0·150^{cB}	0·156^{dB}	0·265^{bA}	0.01
F2	0.08^{aC}	0.133^{bB}	0·165^{abB}	0·178^{bB}	0·187^{cB}	0·365^{aA}	0.01
F3	0.09^{aE}	0.174^{aD}	0·188^{aD}	0·286^{aC}	0·331^{aB}	0·388^{aA}	0.02
F4	0.09^{aE}	0.151^{abD}	0·186^{aC}	0·253^{aB}	0·263^{bB}	0·395^{aA}	0.02

[a] Days of curing process (0, 7 and 19)
[b] Days of storage (0, 30, 60 and 90)
* Standard error of the means (n=9)
Different small letters in the same column indicate significant differences at P<0.05 (Fisher's test). Different capital letters in each row for each parameter indicate significant differences at P<0.05 (Fisher's test). Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl. 25% KCl and 25% $CaCl_2$.

Composition of Fatty Acids

Fatty acid content (Table 2) was tracked during the manufacture and storage of reduced NaCl salamis. Fatty acid composition may be directly affected by lipid-oxidation reactions, and unsaturated fatty acids are the most susceptible to these changes (FENNEMA, 1993). In general, we observed that the content of saturated fatty acids (SFA), monounsaturated (MUFA) fatty acids, and polyunsaturated fatty acids (PUFA) decreased after 30 days of storage, especially in the treatments where NaCl was reduced and replaced by KCl (F2), $CaCl_2$ (F3), and a blend of KCl and $CaCl_2$ (F4). The reduction in unsaturated fatty acids over time indicates that higher lipolytic activity may be occurring in salamis where NaCl content has been replaced by other chloride salts. According to COUTRON-GAMBOTTI et al. (1999), during the processing of fermented products, lipid oxidation leads to decreased unsaturated, long-chain fatty acids and the increased generation of free - fatty acids and phospholipids. For instance,

QUINTANILLA et al. (1996) found higher free fatty acid levels in dry fermented sausages produced with 1.5% NaCl and 1% KCl, compared with samples containing 3% NaCl. In our study, the reduction of unsaturated fatty acids as well as the high levels of TBARS observed mainly in the treatments containing $CaCl_2$ suggest the high lipolytic activity of these salts during the storage of salamis.

The fatty acids predominately found in salamis were palmitic (C16:0), stearic (C18:0), oleic (C18:1(n-9)) and linoleic (C18:2(n-6)). These fatty acids are commonly found at high concentrations in salamis (CASABURI et al., 2007, SÁNCHEZ et al., 2011, ESSID and HASSOUNA, 2013). At each timepoint, some differences between the control group and treatments were observed. These differences can be attributed to high heterogeneity, which is typical in salamis (CAMPAGNOL et al., 2012), since the processing conditions and raw meat material used were the same for all the treatments. At the initial timepoint (0 days), salamis produced with 50% NaCl (F1) showed a significantly lower elaidic acid content (C18:19t) than the control group, and no differences with the control group were observed at 7 days, 19/0 days and 90 days.

At 30 days of storage, minor values of C14:0 (myristic acid) and C16:0 (palmitic acid) were observed compared to with the control group, while minor values of C18:1 (n-9) (oleic acid) and C18:2 (n-6) (linoleic acid) were observed at 60 days of storage compared with the control group. For salamis with 50% added $CaCl_2$ (F3), only eicosatrienoic acid (C20:3 (n-6)) showed a higher value the control group, while heptadecenoic acid (C17:1) and elaidic acid (C18:19t) showed lower values ($P<0.05$) at 60 days.

In the evolution of fatty acids, differences were observed for salamis with 50% added KCl (F2) and 25% KCl and 25% $CaCl_2$ (F4). Treatment F2 differed from the control group ($P<0.05$) owing to its lower content of saturated and monounsaturated fatty acids, such as C14:0, C18:0, C16:1, and C18:1(n-9), among others. On the contrary, treatment F4, compared with the control group, had higher values of unsaturated fatty acids C18:2 (n-6), C20:2, and C20:4 (n-6) ($P<0.05$) and these differences predominated at maturation (19/0 days).

Table 2- Content of fatty acids in the lipid fraction (mg / g fat) during curing process and storage o dry fermented sausages with NaCl reduced or replaced by KCl and/or $CaCl_2$.

Days	Fatty acids	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C14:1	C16:1	C17:1	C18:1 9t	C18:1(n-9)	C20:1(n-9)	C18:2(n-6)	C18:3(n-3)	C20:2	C20:3(n-6)	C20:4(n-6)
	Control	12.0	2.1^{A}	215.7^{b}	4.1^{AB}	121.3	2.3^{bA}	0.7^{AB}	16.9^{AB}	2.7^{AB}	1.3^{ab}	367.4^{abA}	7.4^{abAB}	139.4^{bA}	6.3^{AB}	5.5^{bA}	1.0	4.0^{A}
	F1	11.9	1.8^{A}	237.9^{abA}	3.5	119.2	2.3^{bA}	0.8	15.1	2.6	0.8^{c}	340.2^{abA}	7.3^{ab}	140.7^{b}	7.3^{A}	6.0^{ab}	1.1	4.0
0[a]	**F2**	12.7	1.7^{AB}	257.3^{aA}	3.8^{AB}	127.0^{AB}	2.9^{aA}	0.9^{A}	14.7^{AB}	2.5^{BC}	1.4^{a}	377.9^{aA}	8.3^{aA}	165.8^{aA}	8.0^{A}	6.8^{aA}	1.3^{A}	3.4^{B}
	F3	11.8^{A}	2.1^{A}	222.3^{bABC}	3.8^{AB}	110.2^{ABC}	2.2^{bA}	0.8	16.3^{A}	2.4^{BC}	1.2^{ab}	329.6^{bAB}	6.5^{bBCD}	133.3^{bB}	7.8^{A}	5.4^{bBC}	1.1^{BC}	3.7^{A}
	F4	11.2^{AB}	1.7^{A}	217.3^{bBC}	4.0^{AB}	110.3^{B}	2.3^{bAB}	0.8^{BC}	15.1^{AB}	2.5^{AB}	1.0^{bc}	334.6^{abB}	7.2^{ab}	145.0^{bA}	7.3^{A}	6.1^{abA}	1.2^{ABC}	3.7^{AB}
	Control	12.5	2.1^{A}	246.7	4.2^{AB}	119.9	2.1^{AB}	0.8^{A}	18.3^{A}	3.0^{A}	1.1	372.1^{A}	7.7^{A}	139.9^{bA}	7.1^{A}	6.0^{A}	1.4	3.9^{AB}
	F1	11.9	1.8^{A}	242.4^{A}	4.0	122.5	2.3^{A}	0.8	16.7	2.7	0.8	372.3^{A}	7.6	146.5^{ab}	7.1^{AB}	6.2	1.1	3.4
7	**F2**	13.0	2.0^{A}	258.3^{A}	4.1^{AB}	131.4^{A}	2.0^{B}	0.9^{A}	17.0^{A}	3.4^{A}	1.3	396.6^{A}	7.7^{AB}	161.9^{aA}	8.2^{A}	7.0^{AB}	1.3^{A}	4.1^{A}
	F3	12.0^{A}	1.7^{AB}	238.6^{AB}	4.3^{A}	122.6^{AB}	2.2^{A}	0.8	16.7^{A}	3.0^{A}	1.2	359.0^{A}	7.4^{AB}	146.5^{abA}	7.4^{A}	6.1^{AB}	1.2^{B}	3.5^{A}
	F4	13.0^{AB}	1.9^{A}	254.7^{AB}	4.4^{A}	127.9^{AB}	2.2^{AB}	0.8^{AB}	17.0^{A}	2.9^{A}	1.1	372.1^{B}	8.1	158.9^{aA}	8.0^{A}	6.8^{A}	1.0^{BC}	3.9^{A}
	Control	12.5^{ab}	1.8^{abA}	245.6^{ab}	4.4^{A}	125.1^{b}	2.0^{AB}	0.9^{A}	17.4^{aAB}	2.9^{aA}	0.8	372.5^{bA}	7.8^{A}	147.0^{bA}	7.0^{abA}	6.1^{bcA}	1.0^{ab}	2.2^{bBC}
	F1	12.0^{bc}	1.8^{abA}	241.7^{bA}	4.2	122.4^{b}	2.2^{A}	0.8	16.9^{ab}	2.8^{ab}	0.9	369.3^{bcA}	7.3	150.5^{b}	6.9^{bAB}	5.8^{c}	1.1^{ab}	3.5^{ab}
19/0[b]	**F2**	10.3^{c}	1.4^{bAB}	209.7^{bB}	3.9^{AB}	105.8^{cC}	2.1^{B}	0.7^{AB}	14.1^{bAB}	2.3^{bBC}	1.0	322.4^{cAB}	6.7^{BC}	145.6^{bAB}	6.7^{bB}	5.7^{cA}	1.1^{abA}	3.0^{abBC}
	F3	12.6^{abA}	1.6^{abB}	247.0^{abA}	4.5^{A}	125.8^{bA}	2.4^{A}	0.9	16.9^{abA}	2.7^{abAB}	1.6	376.0^{bA}	8.0^{A}	150.9^{bA}	7.5^{abA}	6.5^{abA}	0.8^{bC}	3.4^{abA}
	F4	14.1^{aA}	2.1^{aA}	284.9^{aAB}	4.7^{A}	144.8^{aA}	2.4^{A}	1.0^{A}	18.5^{aA}	3.0^{aA}	1.0	435.4^{aA}	6.2	188.7^{aA}	8.5^{aA}	7.2^{aA}	1.3^{aABC}	4.0^{aA}
	Control	10.3^{a}	1.0^{B}	194.4^{ab}	3.3^{B}	107.9^{ab}	2.1^{AB}	0.6^{B}	13.3^{abC}	2.1^{B}	1.3	299.1^{abAB}	6.3^{BC}	121.8^{AB}	5.8^{AB}	5.0^{AB}	1.0	2.1^{C}
	F1	9.0^{b}	1.0^{B}	152.6^{bB}	3.2	116.9^{a}	1.9^{AB}	0.6	15.8^{a}	2.1	1.4	348.5^{aA}	6.0	111.7	5.3^{AB}	4.6^{B}	1.1	2.1
30	**F2**	10.5^{a}	0.8^{C}	208.3^{aB}	3.3^{B}	104.0^{abC}	2.0^{B}	0.4^{B}	14.4^{abAB}	2.4^{BC}	1.2	320.4^{abAB}	6.4^{BC}	128.0^{B}	6.1^{BC}	5.0^{C}	0.6^{B}	2.8^{C}
	F3	10.6^{aB}	1.1^{B}	189.5^{abC}	3.5^{B}	94.1^{abC}	1.7^{B}	0.7	13.0^{abB}	2.1^{C}	0.9	279.6^{bB}	5.4^{CD}	112.7^{C}	5.3^{AB}	4.4^{C}	1.0^{BC}	2.2^{B}
	F4	9.0^{abB}	1.1^{B}	181.0^{abC}	3.1^{B}	89.9^{bB}	1.5^{C}	0.6^{C}	12.6^{bB}	2.1^{B}	1.0	268.1^{bC}	5.0	106.6^{B}	4.6^{B}	3.7^{B}	0.9^{C}	2.9^{C}
	Control	11.0	0.6^{bB}	228.9^{ab}	3.4^{AB}	113.2	1.5^{bB}	0.8^{AB}	15.3^{BC}	2.1^{abB}	1.6	342.9^{abAB}	6.7^{ABC}	125.8^{abB}	6.1^{AB}	4.4^{B}	1.1^{b}	2.4^{abC}
	F1	10.8	0.6^{bBC}	180.5^{cB}	3.4	113.0	1.4^{bB}	0.7	14.4	2.3^{ab}	1.4	264.5^{cB}	6.0	102.8^{c}	5.4^{AB}	4.9	1.1^{b}	2.5^{ab}
60	**F2**	10.4	0.7^{bC}	207.6^{bcB}	3.3^{B}	106.7^{BC}	1.1^{bB}	0.7^{AB}	13.5^{B}	2.2^{abBC}	0.8	307.2^{bcB}	5.4^{C}	129.2^{bB}	5.1^{C}	4.7^{C}	1.2^{bA}	2.0^{bD}
	F3	11.8^{B}	0.6^{bC}	233.7^{abAB}	3.8^{AB}	118.1^{AB}	1.5^{bB}	0.7	15.3^{B}	1.8^{bC}	1.3	330.7^{abB}	7.1^{ABC}	141.8^{abC}	6.0^{AB}	5.3^{C}	1.8^{aA}	2.3^{bB}
	F4	13.0^{AB}	0.9^{aBC}	262.3^{aAB}	4.2^{A}	130.0^{AB}	1.6^{aB}	0.8^{AB}	17.0^{A}	2.7^{aB}	1.9	379.8^{aC}	7.8	161.7^{aA}	7.0^{A}	6.1^{A}	1.7^{aAB}	3.6^{aABC}

90	**Control**	12.9	0.4^{B}	240.3	3.6^{AB}	110.0	0.9^{C}	0.8^{A}	15.2^{BC}	2.1^{B}	1.6	332.5^{AB}	5.4^{C}	140.0^{AB}	5.6^{abcB}	4.4^{bB}	1.3	2.8^{BC}
	F1	11.8	0.3^{C}	236.4^{A}	3.6	112.1	1.1^{B}	0.7	15.4	2.5	1.4	328.0^{A}	5.7	126.3	5.0^{bcB}	4.6^{b}	1.1	2.9
	F2	12.1	0.5^{C}	258.7^{AB}	4.3^{A}	124.1^{ABC}	2.0^{B}	0.8^{A}	17.0^{A}	2.8^{B}	1.7	373.2^{A}	7.4^{AB}	161.4^{A}	7.8^{aA}	6.8^{aA}	1.4^{A}	3.2^{BC}
	F3	10.7^{B}	0.4^{D}	210.3^{BC}	3.4^{B}	105.2^{BC}	1.8^{B}	0.7	14.4^{AB}	2.3^{BC}	0.9	308.2^{B}	6.0^{CD}	126.0^{BC}	4.0^{cB}	5.1^{abC}	1.0^{BC}	2.8^{AB}
	F4	12.2^{B}	0.5^{C}	234.8^{AB}	4.2^{A}	114.5^{B}	1.6^{BC}	0.8^{AB}	15.8^{AB}	2.7^{A}	1.3	315.0^{C}	7.0	154.9^{A}	7.5^{abA}	7.0^{aA}	1.4^{AB}	3.0^{BC}
	SEM*	0.4	0.1	0.2	5.8	0.5	0.2	0.1	3.0	0.1	8.5	3.4	0.1	0.3	0.2	0.2	0.1	0.3

[a] Days of curing process (0, 7 and 19)

[b] Days of storage (0, 30, 60 and 90)

* Standard error of the means.

Different capital letters in the same row indicate significant differences at P <0:05 (Fisher's test) between treatments. Different small l letters in each row for each parameter indicate significant differences at P <0:05 (Fisher's test) along the manufacturing and storage. Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl, 25% KCl and 25% $CaCl_2$.

Effects of Salts on Color

The color of salamis is one of the sensory properties influenced by the reduction or replacement of NaCl (GIMENO et al., 2001, CAMPAGNOL et al., 2012). In this study, differences in the color parameters L*, a*, and b* as well as Whiteness were observed in the modified salamis compared with the control group (Table 3). During storage, F1 and F2 showed no difference for the L* value. However, the L* values of salamis in F3 and F4 decreased over time, differing from the control group both for the final product (19/0 days) and at the end of their storage (90 days) ($P<0.05$). ALIÑO et al. (2010) found lower L* values for cured loins with 20% $CaCl_2$ and 10% $MgCl_2$ added. From 60 days of storage, salamis produced with 50% NaCl (F1) had a higher L* value compared with the control group; this higher value was maintained until the end of storage.

Salamis showed no differences in a* values for the final product (19/0d); however, reformulated salamis had higher a* values from the timepoint of 60 days of storage, differing significantly from the control group. This finding can be explained by the negative effect of NaCl on the color of cured meat products during storage. According to SAKATA and NAGATA (1992), NaCl interferes with heme pigment content; the higher the NaCl content, the lower the heme pigment content and, consequently, the lower the red intensity.

Yellow (b*) is one of the color parameters possibly related to lipid oxidation in salamis TOLDRÁ (2006). In this study, the replacement of NaCl interfered with the intensity of the yellow color in salamis during storage for F3 (50% NaCl and 50% $CaCl_2$) and F4 (50% NaCl, 25% KCl, and 25% $CaCl_2$). These treatments displayed an increase in yellow intensity after 90 days of storage. This result suggests that the higher lipid oxidation observed in these treatments may have

adversely interfered with the development of product color (FRANKEL, 1998). The comparison at each analysis timepoint between the control and reformulated salamis allowed us to observe higher b* values ($P<0.05$) for F1, F3, and F4 salamis at 19/0, 30, and 60 days. At the end of storage, only salamis F3 and F4 differed significantly from the control group in terms of b* values. GIMENO et al. (1999) observed higher b* values in salamis with reduced NaCl upon the addition of a blend of chloride salts (NaCl, 10 g/kg; KCl, 5.52 g/kg; $CaCl_2$, 7.38 g/kg).

A reduction in whiteness was observed during storage for salamis with added $CaCl_2$ (F3 and F4). This result is directly related to the increased lipid oxidation observed in these treatments, since the formation of metmyoglobin due to oxidation during the process may have resulted in a darker color (CHAIJAN et al., 2004) for salamis with added $CaCl_2$.

Table 3. Color instrument (L*, a*, b* and whiteness) during storage of salamis with NaCl reduced or replaced by KCl and/or $CaCl_2$.

		Treatments					
	Days	**Control**	**F1**	**F2**	**F3**	**F4**	**SEM[a]**
L*	**19/0**	50.28^{cd}	51.49^{bc}	49.36^{dB}	53.36^{aA}	52.65^{abA}	0.30
	30	50.84	51.43	51.18^{A}	52.08^{B}	50.22^{B}	0.25
	60	50.34^{b}	51.24^{a}	49.51^{bB}	50.33^{bC}	50.69^{abB}	0.14
	90	50.28^{cd}	51.63^{ab}	50.79^{bcAB}	52.24^{aC}	50.94^{bcB}	0.17
a*	**19/0**	9.97^{AB}	10.38	10.54^{AB}	10.19^{C}	10.41^{B}	0.09
	30	10.35^{bcA}	10.80^{abc}	9.96^{cB}	11.14^{abAB}	11.37^{aA}	0.15
	60	9.57^{cB}	10.27^{b}	9.96^{bB}	10.84^{aB}	10.23^{bB}	0.08
	90	9.96^{dAB}	10.57^{c}	10.90^{bcA}	11.55^{aA}	11.24^{abA}	0.11
b*	**19/0**	6.14^{bc}	6.71^{a}	6.45^{ab}	6.88^{aC}	6.64^{abC}	0.08
	30	6.23^{c}	6.97^{ab}	6.67^{bc}	7.33^{aB}	7.17^{aB}	0.09
	60	6.01^{c}	6.65^{a}	6.26^{b}	7.04^{aAB}	7.07^{aB}	0.07
	90	6.56^{c}	6.53^{c}	6.92^{c}	7.90^{aA}	7.85^{abA}	0.09
Whiteness (%)	**19/0**	48.92^{cd}	49.93^{bc}	47.87^{dC}	51.76^{aA}	51.06^{abA}	0.29
	30	49.37	49.75	49.72^{A}	50.25^{B}	48.43^{B}	0.35
	60	49.07^{ab}	49.70^{a}	48.16^{cBC}	48.68^{bcC}	49.15^{abB}	0.14
	90	48.87^{c}	49.91^{ab}	49.12^{bcAB}	50.23^{aB}	49.06^{bcB}	0.16

[a] Standard error of the means (n=9)

Different small letters in the same row indicate significant differences at $P<0.05$ (Fisher's test). Different capital letters in each column for each parameter indicate significant differences at $P<0.05$ (Fisher's test). Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl. 25% KCl and 25% $CaCl_2$.

Conclusion

The results presented herein indicate that a 50% NaCl reduction in results in a reduction in the lipid oxidation of salamis during the curing process and storage. Furthermore, the replacement of 50% NaCl with 50% $CaCl_2$ and with a blend of KCl and $CaCl_2$ was shown to have a negative effect on the oxidative stability of salamis by increasing TBARS values and modifying fatty-acid composition. In addition, color parameters a* and b* increased, while L* and whiteness decreased. Thus, the results suggest that using $CaCl_2$ as a substitute for NaCl increases the intensity of oxidative reactions in salamis while the addition of KCl can be the best alternative to reduce the NaCl content in fermented meat products.

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CAPÍTULO 5

IS THERE A POTENTIAL CONSUMER MARKET FOR LOW SODIUM FERMENTED SAUSAGES?

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Abstract

The NaCl levels in dry fermented sausages were reduced by 50% or were substituted with KCl, $CaCl_2$, or a blend of KCl and $CaCl_2$ (1:1). The quality, safety, and the potential consumer market of dry fermented sausages were assessed. Neither 50% reduction of the NaCl content nor the substitution of 50% of the NaCl with KCl influenced the fermentation and maturation process. However, when $CaCl_2$ was used as the substitute salt (50%), there was a significant decrease in pH, an increase in the water activity, and a decrease in lactic acid and micrococcus bacterial counts. Overall, the sensory acceptance decreased in dry fermented sausages with reduced sodium content. However, cluster analysis and internal preference mapping revealed potential for commercialization of samples with 50% of the NaCl content substituted with KCl or with a mixture of KCl and $CaCl_2$ (1:1).

Keywords: Sodium chloride reduction; potassium chloride; calcium chloride; dry fermented sausages; consumer study.

Practical Application

Excess consumption of sodium chloride, the primary source of dietary sodium in human diet, is associated with increased blood pressure, cardiovascular disease, and some types of cancer. Fermented sausages are among the meat products, with higher sodium contents. The current results are of major importance for the meat industry, once they demonstrate that the low sodium fermented sausages can potentially appeal to certain types of consumers and provide a way forward for future research and product development.

Introduction

Excess consumption of sodium chloride, the main source of sodium in the human diet, is associated with increased blood pressure, cardiovascular disease, and some types of cancer (He and MacGregor 2010; Felicio and others, 2013). For these reasons, over the past few decades, public health bodies and regulatory authorities have established programs to promote the reduction in dietary sodium chloride intake (Food Standard Agency (FSA) 2006; World Health Organization (WHO) 2012), so as to decrease the incidence of chronic diseases related to high sodium levels. Processed food contributes to approximately 80% of sodium consumed by people in industrialized countries (He and MacGregor 2010).

Due to widespread concerns about current eating habits and the struggle to decrease sodium intake, it has become imperative for the meat industry to reduce the sodium content in its products. Of all food products, fermented sausages are among those with highest sodium content. Depending on the formulation, fermentation, and maturation conditions, this type of product may contain approximately 60% of the recommended sodium intake as stated by the World Health Organization (Campagnol and others 2012; World Health Organization (WHO) 2012) in a 50 gram portion.

Sodium chloride (NaCl) is the main source of sodium in dry fermented sausages, and therefore, in order to obtain healthier products, this ingredient must be eliminated or reduced. However, its reduction this is a huge challenge because NaCl significantly affects technological and sensory quality. By solubilizing myofibril proteins, NaCl makes products easier to slice (Barbut 2011). It also influences microbiological stability because reducing the initial water activity favors the development of starter cultures and reduces microbiota

contamination (Fontán and others 2007). These functions of NaCl are essential to produce products that will be stable at room temperature with long shelf life. Furthermore, NaCl is very important for sensory quality. In addition to providing the characteristic salty taste of meat, NaCl also accentuates the taste and flavor of other components and reduces the perception of other stimulants, such as the bitter taste of some compounds (Coultate 2002).

Consequently, research on sodium content reduction in fermented sausages without compromising the technological, microbiological, and sensory qualities, currently focuses on using other chloride salts. Potassium chloride (KCl) is renowned for being safe (Generally Recognized as Safe) and for having an antimicrobial activity similar to that of NaCl (Bidlas and Lambert 2008). Therefore, it is one of the ingredients most often used to reduced the sodium content (Gou and others 1996; Armenteros and others 2009; Cruz and others 2011; Campagnol and others 2012; Dos Santos and others 2014). However, a decline in sensory quality related to emerging bitterness and decreased saltiness have been reported when KCl is used as a sole substitute, constituting the main limitations of its use as a substitute for NaCl in fermented meat products (Gou and others 1996; Gimeno and others 1998; Campagnol and others 2011a; Dos Santos and others 2014). Calcium chloride ($CaCl_2$) is another ingredient that may be used as a NaCl substitute in meat products. Some studies assessed the use of $CaCl_2$ combined with other chloride salts as a strategy to reduce sodium in dry fermented sausages (Gimeno and others 1998, 1999, 2001a; Flores and others 2005; Zanardi and others 2010). In general, these studies report the effect of mono- and divalent salts (KCl, $CaCl_2$ and $MgCl_2$) as salt substitutes on the technological, microbiological and sensory quality of fermented meat products. However, there is little information about the effect of using $CaCl_2$ alone or in

conjunction with KCl on the quality and safety parameters of dry fermented sausages with reduced NaCl content.

Clearly, the collective experience with sodium-reduced fermented sausages shows that these products are not optimum and acceptable to most consumers. Thus, the aim of this study was to assess the quality, safety, and the potential consumer market of dry fermented sausages with 50% of their NaCl content reduced or substituted with KCl, $CaCl_2$, or a blend of KCl and $CaCl_2$ (1:1).

Materials and methods

Dry fermented sausages processing

The NaCl content in the dry fermented sausages was reduced, or 50% of the salt content was replaced by 50% KCl, 50% $CaCl_2$ and a blend containing 25% KCl and 25% $CaCl_2$. The treatments used are listed in Table 1.

The dry fermented sausages were produced using the following main ingredients: pork meat (650 g/kg; moisture: 76.08% ± 0.26, protein: 18.00% ± 0.82, lipids: 4.43% ± 0.10, and ash: 1.07% ± 0.61), beef (200 g/kg; moisture: 77.12% ± 0.22, protein: 18.57% ± 0.12, lipids: 2.20% ± 0.02, and ash: 1.01% ash ± 0.61), and pork back fat (150 g/kg; moisture: 17.28% ± 1.24, protein: 6.54% ± 0.94, lipids: 75.39% ± 0.51, and ash: 0.36% ± 0.55). The raw material was ground with a disk (8 mm) and mixed with the correct amount of NaCl and other ingredients for each treatment described in table 1. The following ingredients were added to the meat mixture in each treatment: glucose (5 g/kg), sucrose (5 g/kg), sodium nitrate (0.15 g/kg), sodium nitrite (0.15 g/kg), sodium ascorbate (0.25 g/kg), white pepper (2 g/kg), garlic (3 g/kg), nutmeg (0.02 g/kg) and starter culture (0.25 g/kg; SPX Floracarn, Chr Hansen). After complete homogenization,

the treatments were stuffed in collagen casings (diameter of 60 mm), and they were cut into slices of approximately 15 cm in length. In total, 60 pieces (approximately 300 g each) were prepared for each treatment. After being stuffed, the samples were subjected to a bath containing a 20% solution of potassium sorbate, and the samples were then ripened in a laboratory ripening cabinet (Menoncin, Erechim, Brazil). The temperature and relative humidity (Tº/UR%) were set as follows: first day, temperature 25°C/95%; second day, 24°C/93%, third day, 23°C/90%, fourth day, 22°C/85%, fifth day, 21°C/80%, sixth day, 20°C/75%, and from the seventh day though the nineteenth day, 18°C/75%. The air speed remained at 5 m/s throughout the processing.

Table 1. Levels of sodium chloride, potassium chloride, and calcium chloride used in dry fermented sausage formulations.

	Treatments (%)				
	Control	**F1**	**F2**	**F3**	**F4**
Sodium chloride (NaCl)	2.5	1.25	1.25	1.25	1.25
Potassium chloride (KCl)	-	-	1.25	-	0.625
Calcium chloride ($CaCl_2$)	-	-	-	1.25	0.625

* Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl, 25% KCl and 25% $CaCl_2$..

Physicochemical analyses

The pH was determined by direct insertion of a pH meter MA 130 (Mettler Toledo Indústria e Comércio Ltd, SP, Brazil). Water activity (Aw) was measured by using a Decagon Aqualab instrument (Decagon Devices Inc., Pullman, USA). The pH and water activity were determined on days 0, 7, and 19 of production. Five pieces of dry fermented sausages from each treatment were used to determine the Aw and pH. Weight loss was defined as the difference in weight of the remaining pieces of meat at sausage formation (day 0) and the product weight at the end of production. Ten pieces of dry fermented sausages per treatment

were used to determine weight loss. Sodium, potassium, and calcium contents were determined at the end of production, according to the method described by AOAC (2005), by using three pieces of dry fermented sausages per treatment.

Texture Profile Analysis (TPA) was performed at the end of the manufacturing process using the Texturometer TA-TX2 (Stable Micro Systems Ltd., Surrey, England) with a load cell of 10 kg. Each sample was cut into 3-cm cylinders and stretched axially into two consecutive circles compressed at 30% with a probe of 30 mm in diameter, moving at a constant test velocity of 1 mm/s. The data were collected, and the texture profile curves were drawn using the program Texture Expert, version 1.11 (Stable Micro Systems Ltd.). The following parameters were calculated: hardness, springiness, cohesiveness, and chewiness. For each treatment, 5 pieces of dry fermented sausages were used in the instrumental texture analysis.

Color was determined at the end of the manufacturing process, using a spectrophotometer-colorimeter model CM-5 (Konica Minolta) with spectral reflectance included as a calibration mode, Standard Illuminant D65, and an observation angle of 10°, while operating in the system CIE (L*a*b*). The values of L* (luminosity), a* (intensity of red), and b* (intensity of yellow) were determined. Five pieces of dry fermented sausages were used per treatment to determine color, with the color parameters being assessed for each sausage at four points in the center of five slices.

Microbiological analyses

The microbiological characteristics of dry fermented sausages were assessed on days 0, 7, and 19 of manufacturing, according to the methodology described by Downes and Ito (2001) in triplicate. Aliquots of 25 grams were

collected, homogenized with 225 mL of 0.1% peptone water (Oxoid Unipath Ltd., Basingstoke, Hampshire, UK), and serially diluted to a decimal scale. Lactic acid bacteria (LAB) were quantified using De Man Rogosa Sharpe agar (Oxoid; 37°C/48 h), aerobic mesophyll bacteria on a standard agar medium for counting (Oxoid; 35°C/48 h), micrococcus bacteria on manitol salt agar (Oxoid;37°C/48 h), total coliforms on violet red neutron-bile crystal agar (Oxoid; 37°C/24 h), and fecal coliforms bacteria in EC broth (Oxoid; 45°C/48 h).

Consumer study

The study protocol was approved by the Research Ethics Committee of the University of Campinas under number 130260. Two sensory tests with consumers were applied on different dates at University of Campinas, in the Sensory Analysis Laboratory, Department of Food Technology, in the Faculty of Food Engineering. A consumer acceptability testing was performed using a non-structured nine-point hedonic scale (1 = dislike extremely to 9 = like extremely (Morais and others 2014a)). The color, aroma, flavor, and texture of the samples were assessed via 196 dry fermented sausage consumers (Meilgaard and others 2006), with 53% being female and 47% male, ranging in age from 18- to 54-years old. In the second consumer test, the JAR and overall acceptability sensory test were used with 106 dry fermented sausage consumers, with 57% being women and 43% being men, ranging in age from 18 to 54 y (Esmerino and others 2013, Paixão and others 2014). The overall acceptability sensory was performed using a non-structured nine-point hedonic scale (1 = dislike extremely to 9 = like extremely). JAR questions were answered on a nine point scale, where 1–4 was extremely less than optimal, 5 was optimal, and 6–9 was extremely more than

optimal. This scale was used to assess salty flavor and the texture of dry fermented sausages (Canto and others 2014). In both the tests, samples were assigned a three-digit code and were evaluated by each consumer in a monadic order, and the order of presentation followed a complete balanced design as described by Stone and others (2012). No additional information concerning the samples was provided to the consumers, in order to prevent errors (Thompson and others 2009). After tasting each sample, all participants were asked to eat a cream-cracker biscuit and drink water. The consumer study was performed in normalized booths under fluorescent lighting.

Statistical analysis

Three independent manufacturing curing times for the fermented sausages were carried out with the same formulation and technology. In each manufacture, three sample units (dry fermented sausage) were taken per sampling day (n = 9). All analyses were performed by triplicate. The results reported in this study are the mean obtained from all the data recorded for each parameter analyzed.

The results of the physicochemical and microbiological analyses and those of the consumer test were analyzed using an analysis of variance (ANOVA) test, and the mean values were compared using the Tukey post-test, with a significance level of 5% ($P \leq 0.05$). Penalty analysis was performed on overall liking scores based on JAR question responses; Penalty analysis is but one of several methods used throughout the marketing research industry to reach conclusions related to the effects of a JAR variable on a different product measure (Drake and others 2011, Gaze and others *in press*). Agglomerative

hierarchical clustering was used to cluster consumer segments, using a dissimilarity matrix with Euclidean distance with Ward's method (Santos and others 2013). An ANOVA was again performed on these overall liking scores to see if differences existed among the consumer clusters, in which cluster and treatment were fixed effects and consumers were random effects.

Internal preference mapping was performed by the principal component analysis (PCA) on the correlation matrix of consumers by products. Internal preference maps transformed consumer acceptance scores into a set of preference dimensions that represent the differences among the samples. Individual acceptance scores are represented by vectors that show the individual directions of increasing preference (Gomes and others 2011). In this method, PCA was first applied on the consumer data in order to interpret the consumer response about the different products. This helped obtain a PCA scores plot and a PCA loadings plot, with the samples as scores and the individual consumer preferences as loadings. Next, all of the sensory attributes were regressed onto the estimated PCA scores from the consumer data, using the linear model (Naes and others 2010).

All the analyses were performed using the software XLSTAT 2013 for Windows (Adinsoft, Paris, France).

Results and discussion

Physicochemical analyses

A decrease in Aw and the eventual dehydration throughout the process contribute to the stability and safety of fermented meat products, especially dry fermented sausages (Toldrá 2006). In this study, Aw values decreased from

0.978–0.968 (day 0) to 0.915–0.887 at the end of the manufacturing process (Table 2). A higher Aw was observed during the processing of dry fermented sausages produced with 1.25% NaCl (F1- 50% NaCl). This result is consistent with that of Toldrá, Flores, and Sanz (2001), who explained that for sodium chloride to preserve and reduce the initial Aw, it must be added at 2 or 3%. After seven days of curing, the products from the treatment with 50% NaCl and 50% $CaCl_2$ (F4) had a higher Aw value than the control ($P \leq 0.05$), and this difference persisted until the end of the manufacturing process. This may be explained by the strong bonding between calcium chloride and the proteins in the meat, which make salt penetration more difficult (Aliño and others 2010).

The lowest pH values (Table 2) at the beginning of the process were those recorded during the treatments involving $CaCl_2$, which were significantly different from that of the control. Gimeno and others (1999, 2001b) previously reported the low pH of dry fermented sausages in the presence of calcium when studying the substitution of NaCl with KCl, $CaCl_2$, and calcium ascorbate. This decrease in pH may have occurred because the pH of the sample dropped when the NaCl concentration increased. It could also have been because when $CaCl_2$, $MgCl_2$, and $ZnCl_2$ were added, the isoelectric point appeared to shift to a lower pH as a result of the anionic effect (von Hippel and Schleich 1969). After the seventh day of manufacturing, the pH continued to decline until the end of the process, and the treatments involving the addition of calcium chloride (F3 and F4) had significantly lower pH values than the control ($P \leq 0.05$).

The weight loss of dry fermented sausages during the manufacturing process due to water loss was approximately 42% (Table 2). A similar result was found by other researchers when studying NaCl substitution using other chloride salts in dry fermented sausages (Flores and others 2005; Campagnol and others

2011a, Campagnol and others 2012). No significant difference was observed between treatments that reduced NaCl content and 50% of replaced the NaCl with KCl and/or $CaCl_2$ and the control.

Decreasing sodium intake as well as increasing potassium and calcium in the diet helps regulate blood pressure (Sacks and others 1998). In this study, reducing sodium and increasing potassium and calcium makes the reformulated sausages a healthier option. The control dry fermented sausages had a sodium concentration of 1729.70 mg/100 g (Table 2). As expected, substituting NaCl with KCl and/or $CaCl_2$ reduced the overall sodium chloride content of the reformulated sausages by 42%. A 50 g portion of the modified sausages would account for 18% of the total daily sodium recommended intake for a healthy diet (WHO 2012) in comparison to approximately 60% from regular sausages. These findings are in accordance with those of the studies on the reduction of NaCl and the addition of other chloride salts in dry fermented sausages (Campagnol and others 2011a) and mortadella (Horita and others 2011). Dry fermented sausages produced with a 50% reduction in NaCl and addition of 50% (F2) or 25% KCl (F4) had 135% and 75% increased potassium, respectively. The dry fermented sausages with added calcium chloride contained 432.13 mg/100 g (F3; 50% $CaCl_2$) and 293.93 mg/100 g (F4; 25% KCl and 25% $CaCl_2$) calcium, which represents, for a 50 g portion of dry fermented sausages, 21.5% and 14.65%, respectively, of the recommended daily intake of calcium for adults (1000 mg) (Ross and others 2011).

The texture profile evaluation (Table 2) showed that a 50% reduction in NaCl and the addition of potassium and calcium chloride did not change the texture of the dry fermented sausages. The dry fermented sausages produced with 50% NaCl and substitution of 50% NaCl with KCl and/or $CaCl_2$ (F2, F3, and

F4) did not significantly differ from the control with regard to the hardness, springiness, cohesiveness, and chewiness. Consistent with these results, Campagnol and others (2011b) did not encounter differences in hardness, cohesion, or chewiness in dry fermented sausages with 50% NaCl substituted with KCl. Similar results were also reported by Dos Santos and others (2014) and Aliño and others (2010). However, the treatment with a 50% NaCl reduction (F1) had a lower chewiness value when compared with the other treatments ($P \leq 0.05$). According to Barbut (2011), the simple reduction in the concentration of sodium chloride during dry fermented sausage production may affect the extraction and solubilization of myofibril proteins and hence hinder texture development and sliceability of the product.

The color of dry fermented sausages with a reduced salt content may be altered when substituting NaCl with KCl and $CaCl_2$. In this study, the color parameters, luminosity (L*), red intensity (a*), and yellow intensity (b*) were measured at the end of the manufacturing process (Table 2). The addition of potassium and calcium chloride did not affect the L* of reformulated dry fermented sausages. The a* values were higher than those of the control ($P \leq 0.05$) in the treatments with 50% KCl (F2) or 25% KCl and 25% $CaCl_2$ (F4).Conversely, the values of b* were significantly higher than those of the control in treatments with 50% (F3) and 25% $CaCl_2$ (F4). The differences observed may be attributed to the fact that the typical fermented meat product color is rather heterogeneous (Campagnol and others 2011a). This was also observed by Gimeno and others (2001b) when NaCl was partially substituted by calcium ascorbate in dry fermented sausages, observing higher values for a* and b*, whereas Gimeno and others (1999) observed higher L* and b* values in dry fermented sausages with reduced NaCl, upon the addition of a blend of chloride

salts (NaCl, 10 g/kg; KCl, 5.52 g/kg; $CaCl_2$, 7.38 g/kg), even when using an equivalent ionic force.

Table 2. Physicochemical properties (± standard deviation) of dry fermented sausages with 50 NaCl substituted with KCl and/or $CaCl_2$.

	Days*	Control**	F1	F2	F3	F4
Aw	**0**	0.968 ± 0.00^{b}	0.978 ± 0.00^{a}	0.973 ± 0.00^{b}	0.973 ± 0.00^{b}	0.972 ± 0.00^{b}
	7	0.942 ± 0.01^{b}	0.960 ± 0.00^{a}	0.942 ± 0.00^{b}	0.952 ± 0.00^{a}	0.942 ± 0.01^{b}
	19	0.882 ± 0.01^{b}	0.915 ± 0.00^{a}	0.873 ± 0.02^{b}	0.907 ± 0.01^{a}	0.887 ± 0.01^{b}
pH	**0**	5.78± 0.04^{b}	5.85 ± 0.02^{a}	5.92 ± 0.11^{a}	5.29 ± 0.14^{d}	5.51 ± 0.07^{c}
	7	4.67 ± 0.03^{a}	4.64 ± 0.03^{a}	4.68 ± 0.04^{a}	4.42 ± 0.05^{c}	4.54 ± 0.02^{b}
	19	4.81± 0.06^{ab}	4.76 ± 0.05^{b}	4.85 ± 0.04^{a}	4.51 ± 0.04^{d}	4.69 ± 0.06^{c}
Weight loss (%)	**19**	41.52 ± 1.47^{a}	41.53 ± 1.95^{a}	42.47 ± 2.07^{a}	42.18 ± 0.85^{a}	42.64 ± 0.73^{a}
Sodium (mg/100g)	**19**	1729.70 ± 2.87^{a}	1021. 43 ± 2.50^{b}	933.07 ± 3.05^{b}	1021.13 ± 2.39^{b}	1006.43 ± 2.72^{b}
Potassium (mg/100g)	**19**	795.51 ± 2.10^{d}	776.41 ± 3.21^{d}	1870.37 ± 3.81^{a}	873.82^{c} ± 3.02^{c}	1389.98± 3.64^{b}
Calcium (mg/100g)	**19**	12.63 ± 0.95^{c}	11.37 ± 0.30^{c}	9.71 ± 1.09^{c}	432.13 ± 2.81^{a}	293.93 ± 1.69^{b}
Hardness (N)	**19**	34.90 ± 2.99^{ab}	29.71 ± 5.07^{b}	40.07 ± 9.81^{a}	35.47 ± 7.18^{ab}	41.74 ± 9.07^{a}
Elasticity (mm)	**19**	0.63 ± 0.04^{a}	0.66 ± 0.03^{a}	0.63 ± 0.03^{a}	0.63 ± 0.02^{a}	0.66 ± 0.03^{a}
Cohesiveness	**19**	0.64 ± 0.02^{a}	0.64 ± 0.01^{a}	0.64 ± 0.03^{a}	0.66 ± 0.03^{a}	0.64 ± 0.03^{a}
Chewiness (N)	**19**	14.96 ± 2.25^{a}	12.00 ± 2.34^{b}	16.33 ± 5.57^{a}	15.56 ± 3.21^{a}	17.70 ± 4.10^{a}
L	**19**	48.91± 0.58^{ab}	49.21 ± 0.70^{a}	46.77 ± 0.92^{b}	49.39 ± 0.95^{a}	49.72 ± 1.09^{a}
a*	**19**	11.88 ± 0.65^{b}	13.03 ± 0.60^{ab}	13.85 ± 0.67^{a}	12.99 ± 0.57^{ab}	13.50 ± 0.78^{a}
b*	**19**	7.20 ± 0.37^{b}	7.28 ± 0.32^{b}	7.68 ± 0.25^{ab}	8.20 ± 0.30^{a}	8.15 ± 0.39^{a}

* Curing times

** Means ± standard deviation

Means in the same line with the same lower case letter are not significantly different according to a Tukey post-test ($P \geq 0.05$). Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl, 25% KCl and 25% $CaCl_2$.

Microbiological analyses

The growth of microorganisms in fermented sausages is directly related to dehydration, fermentation of carbohydrates, and acidification (Fontán and others 2007). Lower counts were observed for aerobic mesophyll microorganisms and lactic acid bacteria (Table 3) in treatments involving $CaCl_2$ addition at the beginning of the process (day 0). However, at the end of processing (19 days), there was no difference between treatments with reduced NaCl and the control.

The micrococcus count at the beginning of processing ranged from 5.99 to 5.47 log UFC/g. A lower count was observed in the treatment with 50% $CaCl_2$ (F3), which significantly differed from that of the control. This may be attributed to the lower initial pH (5.29) of the dry fermented sausages produced with 50% $CaCl_2$, as micrococcus bacteria are sensitive to acidic environments (Fontán and others 2007). The evolution of micrococcus growth during the manufacturing process is characterized by microbial count reduction over time, mainly due to the acidification and dehydration of the products. In this study, this was observed, and at the end of the manufacturing process (19 days), the dry fermented sausages had microbial counts of approximately 4 log UFC/g. A lower micrococcus count (Table 3) was observed in treatments with 50% $CaCl_2$ (F3) or 25% KCl and 25% $CaCl_2$ (F4), which significantly differed from that in the control. A lower micrococcus count may have been affected by the pH (Gimeno and others 2001a) in treatments F3 and F4, with values of 4.51 and 4.69, respectively. Gimeno and others (1998) achieved similar results when studying the replacement of NaCl with KCl, $MgCl_2$, or $CaCl_2$ in dry fermented sausages. Micrococcus bacteria in fermented sausages reduce nitrates and nitrites, develop color, and give aroma (Gimeno and others 2001a; Fontán and others 2007). In

this study, the lower micrococcus count may have influenced the higher b* value (Table 2) in dry fermented sausages produced with calcium chloride.

The initial count of thermotolerant coliform bacteria on day 0 (Table 3) was approximately 2 log.UFC^{-1}, regardless of the mixture of salts used. Due to the rapid drop in pH and increase in lactic acid bacteria, the thermotolerant coliform bacteria were eliminated after 7 days of curing. No thermotolerant coliform bacteria of fecal origin were detected in any experiment.

Table 3. Microbiological characteristics (log UFC.g^{-1}) of dry fermented sausages with 50% NaCl substituted with KCl and/or $CaCl_2$.

	Days*	Control**	F1	F2	F3	F4
Mesophilic aerobic	**0**	6.16 ± 0.07 ^{b}	6.36 ± 0.07 ^{a}	6.00 ± 0.08 ^{cd}	5.90 ± 0.08 ^{d}	6.03 ± 0.04 ^{c}
	7	7.25 ± 0.02 ^{cd}	7.64 ± 0.03 ^{b}	7.19 ± 0.01 ^{d}	7.98 ± 0.04 ^{a}	7.35 ± 0.02 ^{c}
	19	7.04 ± 0.11 ^{cd}	7.17 ± 0.21 ^{cd}	6.93 ± 0.31 ^{d}	7.57 ± 0.05 ^{a}	7.32 ± 0.21 ^{b}
Lactic acid bactéria	**0**	6.12 ± 0.07 ^{b}	6.20 ± 0.04 ^{a}	6.06 ± 0.01 ^{c}	5.89 ± 0.04 ^{d}	5.92 ± 0.07 ^{d}
	7	7.44 ± 0.10 ^{c}	7.75 ± 0.11 ^{b}	7.39 ± 0.30 ^{c}	8.14 ± 0.06 ^{a}	7.61 ± 0.09 ^{bc}
	19	7.22 ± 0.13 ^{a}	7.28 ± 0.15 ^{a}	7.14 ± 0.26 ^{a}	7.27 ± 0.01 ^{a}	7.19 ± 0.12 ^{a}
Micrococcaceae	**0**	5.94 ± 0.08 ^{a}	5.99 ± 0.08 ^{a}	5.86 ± 0.19 ^{a}	5.47 ± 0.06 ^{b}	5.96 ± 0.09 ^{a}
	7	5.50 ± 0.04 ^{ab}	5.28 ± 0.09 ^{c}	5.81 ± 0.11 ^{a}	4.19 ± 0.24 ^{e}	4.60 ± 0.29 ^{d}
	19	4.51 ± 0.32 ^{a}	4.27 ± 0.21 ^{ab}	4.35 ± 0.05 ^{ab}	4.09 ± 0.36 ^{b}	4.00 ± 0.01 ^{b}
Total coliforms	**0**	2.09 ± 0.06 ^{b}	2.16 ± 0.10 ^{ab}	2.37 ± 0.10 ^{a}	2.26 ± 0.04 ^{ab}	2.34 ± 0.25 ^{a}
	7	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
	19	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00

* Curing times

**Means ± standard deviation

Means in the same line with the same lower case letter are not significantly different according to a Tukey post-test ($P \geq 0.05$). Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl, 25% KCl and 25% $CaCl_2$.

Consumer study

Consumer acceptance of the dry fermented sausages with 50% of their NaCl content reduced or substituted with KCl, $CaCl_2$, or a blend of KCl and $CaCl_2$ are shown in Table 4. The color and aroma of dry fermented sausages with 50% $CaCl_2$ (F3) obtained were less well-liked than the control. The poorer color ratings of this treatment may be correlated with a higher yellow intensity (b*) observed in the instrumental color assessment of the dry fermented sausages in this study (Table 2). The low acceptance of aroma in the dry fermented sausages produced with 50% $CaCl_2$ (F3) may be associated with higher lipid oxidation, which occurs in fermented sausages with added calcium, according to Flores and others (2005) and Zanardi and others (2010).

During the flavor assessment, consumers gave lower ratings to the treatments with 50% NaCl (F1), 50% KCl (F2), 50% $CaCl_2$ (F3), and 25% KCl/ 25% $CaCl_2$ (F4), as compared with the control. Sensory acceptability tests resulted in lower ratings given by consumers to all of the treatment samples compared to the control, with the 50% $CaCl_2$ replacement receiving the lowest overall acceptability scores. Other researchers have reported low sensory acceptance of other types of meat products with NaCl substituted with $CaCl_2$ (Horita and others 2011; Armenteros and others 2012). The presence of calcium ions may give a bitter, metallic, and astringent taste, which may affect the sensory acceptance of reformulated products (Toldrá 2006). Dos Santos and others (2014) and Campagnol and others (2011b) studied the use of flavor enhancers, such as inosinate and disodium guanylate, and the amino acids lysine and taurine to mellow the sensory defects caused by substituting NaCl with KCl. The results were rather promising and may be an alternative to hide the unpleasantness caused when using calcium chloride as a salt substitute for NaCl.

The textures of dry fermented sausages with 50% reduced NaCl substituted with KCl (F2) and/or $CaCl_2$ (F3 and F4) were rated as less well-liked ($P \leq 0.05$) than the control. However, this difference was not observed in the instrumental analysis of the texture profile in this study (Table 2). This behavior is referred to fermented meat products, as reported by dos Santos et al (2014), and Campagnol et al (2012).

Using JAR scores, no treatment was considered excellent for the analyzed attributes (Table 4). From the sensory standpoint, the acceptance did not reach 70% of the responses in the range of 5 points (Meullenet and others 2007). For salty flavor, values varied from JAR 46.23% (control) to JAR 23.58% (F2 and F3). For texture, these values varied from JAR 52.89% (control) to JAR 32.08 (F3). Penalty analysis indicated that for salty taste, the highest values were recorded for treatments F3 (50% NaCl and 50% $CaCl_2$) and the control, with 2.274 and 1.007 ($P < 0.0001$ and $P < 0.003$, respectively). However, for texture, the value varied between 1.154 ($P < 0.001$, control) and 0.596 ($P < 0.094$, F4; 50% NaCl, 25% KCl, and 25% $CaCl_2$). Generally speaking, the results suggest that the addition of $CaCl_2$ to dry fermented sausages must be done with caution in order to not have a negative impact on the consumer's perception of salty taste. Interestingly, the penalty values for treatments with 50% reduced NaCl substituted with KCl were intermediate, with values between 0.692 and 0.926 for salty taste and 0.596 and 0.846 for texture. This suggests that KCl should be added to mixtures of substitute salts that are used in the dry fermented sausages. Our results generally reinforce the current challenge of reducing the sodium chloride content in meat products, indicating that further strategies are needed to minimize the sensory defects caused by using other chloride salts specifically those with calcium.

Table 4. Consumer study of dry fermented sausages with 50% NaCl substituted with KCl and/or $CaCl_2$.

Sensory acceptance*	Control	F1	F2	F3	F4
Color	7.00^{a} ± 1.43	6.76^{a} ± 1.44	6.93^{a} ± 1.46	6.30^{b} ± 1.62	6.89^{a} ± 1.49
Aroma	5.91^{a} ± 1.99	5.89^{a} ± 1.91	5.68^{ab} ± 1.85	5.21^{b} ± 1.89	5.46^{ab} ± 2.02
Taste	6.60^{a} ± 1.82	6.06^{b} ± 1.77	5.97^{b} ± 1.85	4.57^{d} ± 2.00	5.39^{c} ± 1.99
Texture	6.80^{a} ± 1.64	6.80^{a} ± 1.55	6.33^{b} ± 1.70	5.93^{b} ± 1.81	6.26^{b} ± 1.77
JAR test*					
Salty taste (%)					
Too less	25.47^{c}	38.68^{b}	41.51^{a}	51.89^{a}	41.51^{a}
Just-about-right	46.23^{a}	44.34^{a}	23.58^{b}	23.58^{b}	33.02^{a}
Too more	28.30^{a}	16.98c	34.91^{a}	24.53^{b}	25.47^{b}
Texture (%)					
Too less	17.92^{b}	28.30^{b}	39.62^{a}	45.28^{a}	45.25^{a}
Just-about-right	52.89^{a}	48.11^{a}	39.62^{a}	32.08^{b}	42.45^{a}
Too more	29.35^{a}	23.58^{b}	20.75^{b}	22.64^{b}	12.26^{c}
Overall acceptability**					
Cluster 1 (n= 34)	6,35^{b}	4,85^{b}	5,29^{b}	3,09^{b}	3,47^{c}
Cluster 2 (n=34)	6,65^{b}	7,09^{a}	5,94^{b}	5,32^{a}	5,59^{b}
Cluster 3 (n= 38)	8,03^{a}	6,74^{a}	7,32^{a}	5,27^{a}	7,05^{a}

Means ± standard deviation

*Means in the same line with the same lower case letter are not significantly different according to a Tukey post-test (P ≥ 0.05). Nine-point hedonic scale (1= dislike extremely to 9= like extremely). Just JAR values are displayed as percentages of consumer ratings from options: 1–4 for too less, 5 for just-about-right, and 6–9 for too more. **Means in the same line with the same lower case letter are not significantly different according to LSD test (P ≥ 0.05) between clusters. Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl, 25% KCl and 25% $CaCl_2$.

The internal preference map (Fig. 1) explained 64.70% of the variation in consumer acceptance of dry fermented sausages, with 45.02% and 19.68% in the first and second dimensions, respectively. The first dimension separated the treatments into two groups: 1) control (100% NaCl) and F1 (50% NaCl) and 2) F2 (50% NaCl and 50% KCl). The second dimension separated the treatments into a third group composed of treatments with added calcium chloride (F3 + F4). Most consumers were located to the right side (Fig. 1) of the map providing evidence that the Control, F1 (50% NaCl), and F2 (50% NaCl and 50% KCl) treatments were preferred. These treatments were characterized by the reduction

in NaCl as well as the addition of KCl. On the other hand, treatments F3 (50% NaCl and 50% $CaCl_2$) and F4 (50% NaCl, 25% KCl and 25% $CaCl_2$) were not well accepted by consumers.

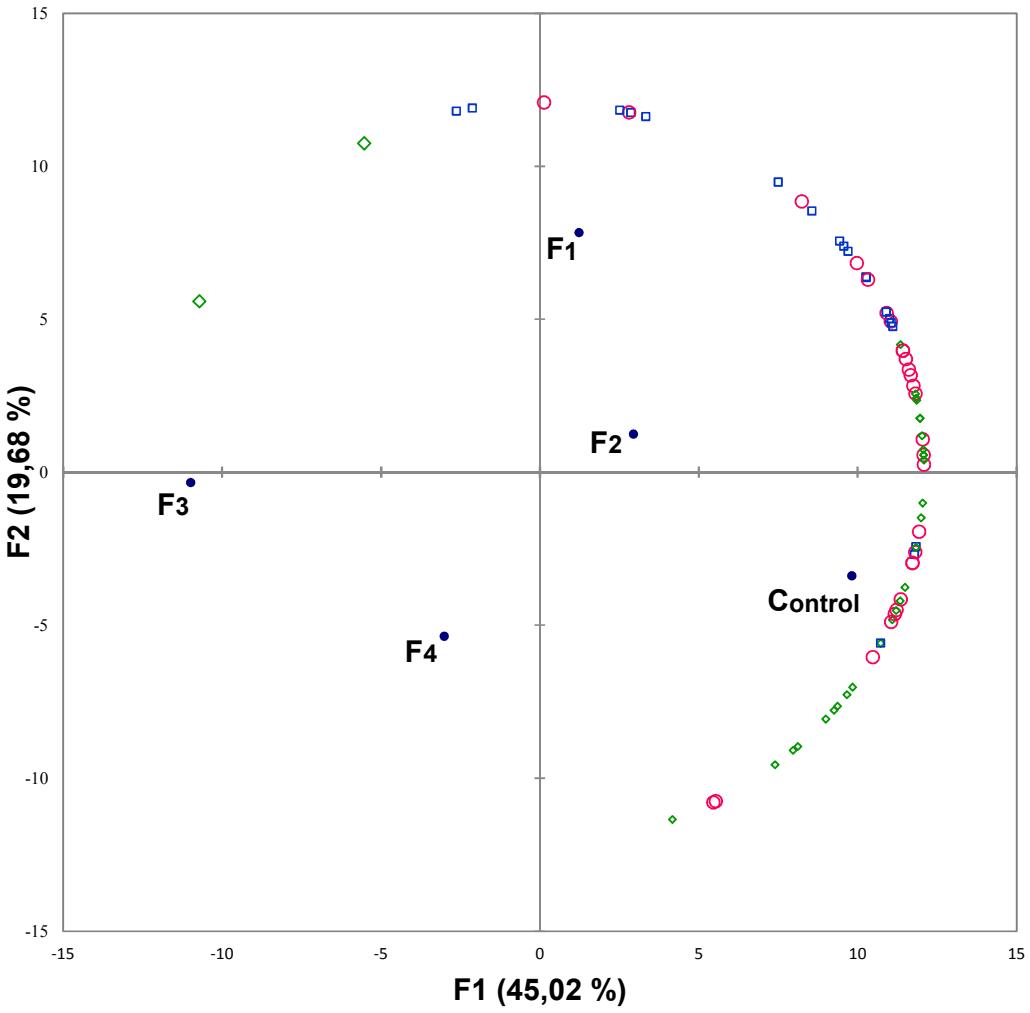

Figure 1. Internal preference map of consumers of dry fermented sausages with 50% NaCl substituted with KCl and/or $CaCl_2$ (n = 106). Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl, 25% KCl and 25% $CaCl_2$.
○- Cluster 1 (n=34), □- Cluster 2 (n= 34) and ◊- Cluster 3 (n= 38).

The resulting dendogram of the hierarchical cluster analysis (HCA, Fig. 2) resulted in three similarly distributed segments, according to the number of people, while Table 4 shows the means overall acceptance values among the

three clusters. Both the first and second cluster grouped 34 individuals, while the third grouped 38 individuals. The control sample had the highest values with regard to the overall acceptance, varying from 8.03 to 6.35 in cluster 1 and 3. However, in segment 2, sample F1 (50% NaCl) had the highest value for overall acceptance, with 7.09 ($P < 0.05$). Generally speaking, the HCA results showed that there is commercialization potential for samples with 50% reduced sodium content in formulation F2 (50% NaCl and 50% KCl) and F4 (50% NaCl, 25% KCl, and 25% $CaCl_2$), showing that there is a consumer market for these treatments. These samples were assessed by a group of consumers (cluster 3). These results will be useful for the meat product industry, where it has been widely reported that the only product that is acceptable to consumers is with a 40% reduction in NaCl (Gou and others 1996). Future studies need to assess the development of the sensory profile of products using descriptive testing, such as quantitative descriptive analysis (Pimentel and others 2013, Morais and others 2014b) and consumer profiling techniques, as projective mapping and ultra flash profiling (Santos and others 2013). Finally, methodologies that involve increased consumption of special foods, such as repeated exposure (Costa and others 2014), should be equally evaluated.

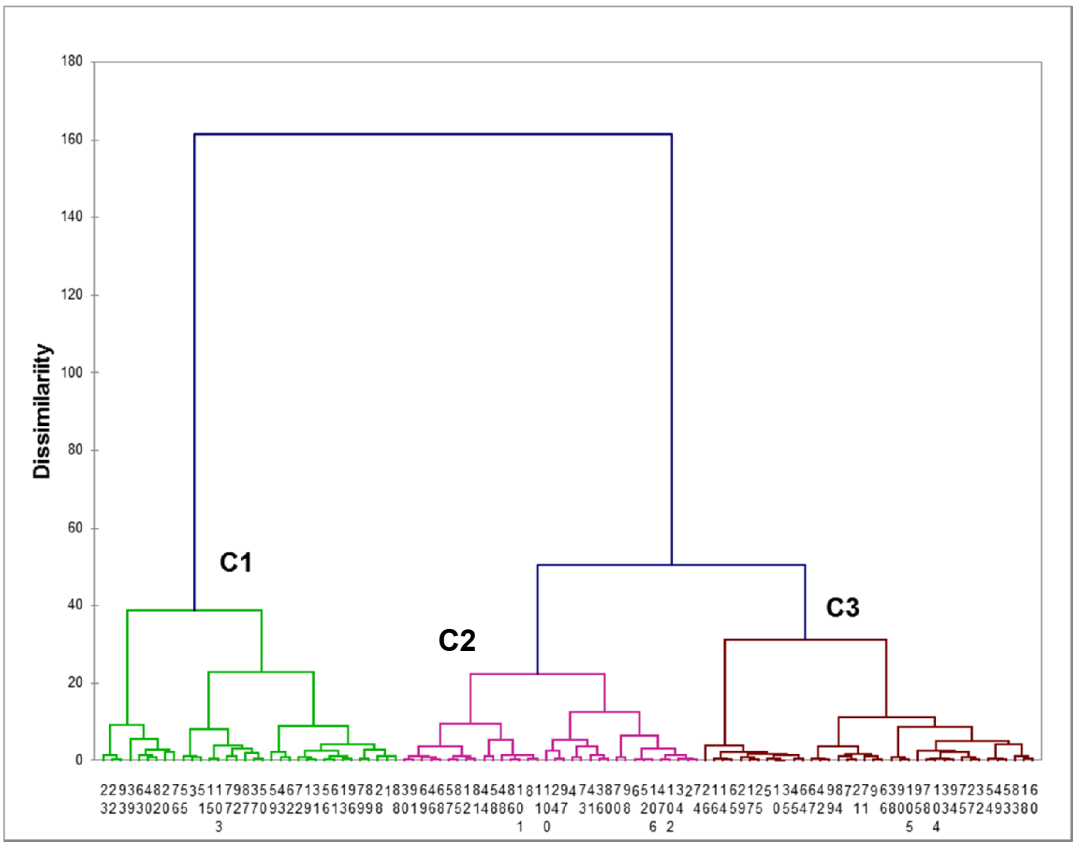

Figure 2. Dendogram of consumers of dry fermented sausages with 50% NaCl substituted with KCl and/or $CaCl_2$ (n = 106). Control- 100% NaCl; F1- 50% NaCl; F2- 50% NaCl and 50% KCl; F3- 50% NaCl and 50% $CaCl_2$; F4- 50% NaCl, 25% KCl and 25% $CaCl_2$. Cluster 1 (n=34), Cluster 2 (n= 34) and Cluster 3 (n= 38).

Conclusion

Reducing sodium chloride and substituting it with KCl and/or $CaCl_2$ makes reformulated dry fermented sausages healthier, reducing the sodium content by approximately 42%. Reducing NaCl by 50% and substituting that 50% with KCl caused small alterations in the technological dry fermented sausage manufacturing process. The 50% replacement of NaCl by $CaCl_2$ negatively affected the manufacturing process of the dry fermented sausages, since the product presented high Aw, low pH, and lower micrococcaceae counts. Generally, dry fermented sausages manufactured with reduced NaCl were less

accepted by the consumers. Cluster analysis and internal preference mapping identified a group of consumers that exists for dry fermented sausages with a 50% reduced NaCl content substituted with KCl or a blend of KCl and $CaCl_2$ (1:1). Thus, the present study suggests a potential consumer market for low-sodium fermented sausages, not an actual market. A marketing study is required to investigate the existence of this market.

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Author Contributions

B.A. dos Santos- collect test data, drafted the manuscript and interpreted the results; P.C.B. Campagnol- Drafted the manuscript and interpreted the results; A.G. da Cruz- performed the statistic analysis and interpreted the results; M. A. Morgano- performed the sodium, potassium, and calcium analysis in dry fermented sausages and interpreted the results; R. Wagner- supervised performance of the experiments and assisted with design and writing; M.A.R. Pollonio- designed the study, supervised performance of the experiments, interpreted the results and assisted with design and writing.

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CAPÍTULO 6

CHECK ALL THAT APPLY AND FREE LISTING TO DESCRIBE THE SENSORY CHARACTERISTICS OF LOW SODIUM DRY FERMENTED SAUSAGES: COMPARISON WITH TRAINED PANEL

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ABSTRACT

The urgent need for sodium reduction in meat products to enable healthy food choices has led food industry to search for more dynamic and fast methodological approaches to assess the sensory characteristics of their products. In the present study, dry fermented sausages with reduction in NaCl, replaced by KCl, $CaCl_2$, and a blend of KCl and $CaCl_2$ were evaluated for their sensory properties using a Check all that apply questionnaire (CATA) and a Free listing task. The results were compared with those of a trained panel using Quantitative Descriptive Analysis (QDA). Absence of concordance was observed between the CATA and Free listing towards the two bidimensional sensory maps and configuration of the samples in comparison to QDA. However, free listing was able to generate a similar and resumed vocabulary when compared to QDA. Our findings suggest the potential of free listing as sensory descriptive methodology in the development of reformulated food products with respect to sodium reduction.

Keywords: Check All That Apply; Free Listing; Quantitative Descriptive Analysis; Dry Fermented Sausages; Sodium Reduction.

1 Introduction

Excessive sodium intake has been widely discussed by public health agencies that, concerned about the increased risk for cardiovascular diseases, have recommended reducing sodium chloride consumption (World Health Organization (WHO), 2012), the primary source of sodium in processed foods. The reduction of sodium chloride can be achieved by reducing or partially replacing NaCl by other chloride salts, thus allowing consumers to adapt to the new flavor intensity (Dotsch et al. 2009). In fermented meat products, reformulation studies using different chloride salts have shown that a reduction of up to 50% NaCl is possible from a technological and microbiological point of view (Campagnol, Santos, Terra & Pollonio, 2012; Dos Santos, Campagnol, Morgano & Pollonio, 2014). However, in hedonic sensory studies with consumers, many authors have reported low acceptance, due to the development of a bitter and metallic taste (Guàrdia, Guerrero, Gelabert, Gou & Arnau, 2008), particularly when KCl is used as a salt substitute (Campagnol et al. 2012; Dos Santos et al. 2014).

Traditionally, the sensory profile of processed food products is developed using quantitative descriptive analysis (QDA). QDA is a standardized methodology involving the evaluation of both the qualitative and quantitative sensory characteristics of the product, which is performed with a team previously assessed for their repeatability and discrimination abilities, subjected to numerous training sessions (Drake, 2007). QDA is time-demanding for its implementation and depends on the availability of a trained panel, in addition to a sensory profile that is unique to a particular class of product (Cadena, Cruz, Netto, Castro, Faria & Bolini, 2013; Morais, Cruz, Faria & Bolini, 2014).

C*heck all that apply* (CATA) has been identified as a promising methodology aimed to establish the sensory profiling of food matrices using consumers (Jorge et al., 2015; Ares, Saldamando, Giménez & Deliza, 2014; Cruz et al., 2013; Ares & Jaeger, 2013) The primary advantage of a CATA questionnaire is that it allows multiple options to be selected, instead of limiting consumers to select only one response or focusing their attention and evaluating specific attributes. CATA questionnaire have been applied to the development of functional yogurts (Cadena et al. 2014, Cruz et al., 2013) and milk desserts (Ares, Barreiro, Deliza, Giménez & Gámbaro, 2010).

Free listing is a technique regularly used in anthropological studies aimed to investigate cultural domain analysis (Rusell Bernard, 2005), which is defined as the study of how people in a group think about lists of things that are somehow related (Hough & Ferraris, 2010). Free listing has been increasingly used in several studies on consumer science, such as reports on the insight in the menus in Argentina (Libertino, Ferraris, López Osornio & Hough, 2012), cross-cultural study to generate consumers' texture vocabulary in Spanish-speaking countries (Antmann, Ares, Varela, Salvador, Coste & Fiszman, 2011), and relevance of package characteristics of milk desserts (Ares & Deliza, 2010) as well as to discover consumers' perception of wellbeing in food-related consumption of wine (Ares et al. 2014). Hough and Ferraris (2010) presented free-listing in a food science audience with the possibility of sensory descriptors would be generated by a trained panel and/or consumers, and frequently listed descriptors would be more relevant to the sample set or product category than those less frequently listed. However, to the best of our knowledge, this study has not been done.

Dry fermented sausages are traditional meat products characterized by high sodium levels (approximately 6% in the final product) and have therefore

been the targets of sodium reduction with a requirement to preserve their sensory properties and consumer acceptance. In this context, the aim of this study was to evaluate the performance of CATA questionnaires and free-listing for establishing a sensory profile of dry fermented sausages with a reduction or replacement of 50% NaCl with KCl, $CaCl_2$, and a *blend* KCl and $CaCl_2$ (1:1). For comparison and reference, the sensory descriptors of the dry fermented sausages were also assessed by a trained panel through the quantitative descriptive analysis method.

2 Materials and Methods

2.1 Dry fermented sausages: Experimental design

The NaCl content of the dry fermented sausages was reduced by 50% or substituted by KCl, $CaCl_2$, or a blend of KCl and $CaCl_2$ (1:1), were prepared as follows: control (2.5% NaCl), 50% salt reduced (1.25% NaCl, F1), 50% replaced by KCl (1.25% NaCl and 1.25% KCl, F2), 50% replaced by $CaCl_2$ (1.25% NaCl and 1.25% $CaCl_2$, F3), and 25% replaced by KCl and $CaCl_2$ (1.25% NaCl, 0.625% KCl and 0.625% $CaCl_2$, F4). In addition, a commercial sample of dry fermented sausage was also used in this study (containing 43.5 g fat, 25 g protein, 1645 mg sodium, and 120 mg calcium/100 g of product according to the label, COM) from a commercial brand marketed in the Brazilian territory. The commercial sample was purchased in a supermarket in the city of Campinas (SP), stored in a cooler with ice, and immediately brought to the laboratory, where it was refrigerated (4 °C ± 1 °C) together with the other samples.

The dry fermented sausages were produced using the primary following ingredients: pork meat (650 g / Kg), beef (200 g / Kg), and pork back fat (150 g / Kg). The raw material was ground with a disk (8 mm) and mixed with the correct

amount of NaCl and other ingredients for each treatment. The manufacturing process was as described by dos Santos et al. (2015a). The following ingredients were added to each meat mixture: glucose (5 g / kg), sucrose (5 g / kg), sodium nitrate (0.15 g / kg), sodium nitrite (0.15 g / kg), sodium ascorbate (0.25 g / kg), white pepper (2 g / kg), garlic (3 g / kg), nutmeg (0.02 g / kg), and starter culture (0.25 g / kg; SPX Floracarn, Chr. Hansen). After complete homogenization, the treatments were stuffed in collagen casings (diameter of 60 mm) and cut into slices of approximately 15 cm in length. In total, 40 pieces (approximately 300 g each) were prepared for each treatment. After being stuffed, the samples were subjected to a bath containing a 20% solution of potassium sorbate, and the samples were then ripened in a laboratory ripening cabinet (Menoncin, Erechim, Brazil). The temperature and relative humidity (Tº / UR%) was set as follows: first day, 25°C / 95%; second day, 24°C / 93%; third day, 23°C / 90%; fourth day, 22°C / 85%; fifth day, 21°C / 80%; sixth day, 20°C / 75%; and from the seventh day to the nineteenth day, 18°C / 75%. The air speed remained at 5 m/s throughout the manufacturing process.

2.2 Quantitative Descriptive Analysis (QDA)

The study protocol was approved by the Research Ethics Committee of the University of Campinas under protocol number 130260. Twenty-five assessors were pre-selected by triangular tests, who found a difference in the salty taste between the dry fermented sausages with different NaCl level. Each assessor participated in nine repetitions, and the assessors with more than 80% correct responses were selected, totaling 18 assessors.

The network method (Moskowitz, 1983) was used at this stage to determine the descriptors for the six samples of dry fermented sausages. The samples were presented in pairs, and each panelist described the similarities and differences with respect to the appearance, aroma, flavor, and texture of the attributes. References were determined by a consensus between all assessors, and they were then trained using the identified references for the product attributes, as shown in Table 1.

The quantification of the sensory descriptors was performed in nine training sessions (one hour, three times a week) with the selected assessors (8 female and 7 male, aged 21-40). The six samples were evaluated in nine repetitions in a monadic form, following a balanced complete block design (MacFie, Bratchell, Greenhoff & Vallis, 1989). A two-way analysis of variance (ANOVA) with two sources of variation (sample and repetition) to each descriptor and each assessor was applied, and the final panel was chosen to participate according to their discriminating capability ($p<0.50$) and repeatability ($p>0.05$) using data collected during the training sessions (Cadena, Cruz, Faria & Bolini, 2012; Cadena et al. 2013). The fifteen selected assessors assessed six samples per session in a total of six sessions. Each evaluator received an assessment form and was invited to rate the intensity of each attribute on a linear scale with 9 cm (unstructured) anchored on the left end by “little” or “none” and on the right end by “very” (Drake, 2007; Stone, Bleibaun & Thomas, 2012).

2.3 Check all that apply (CATA)

One-hundred and six consumers (43% male, 57% female, aged 18-55) were asked to complete a check-all-that-apply (CATA) questionnaire with 15

terms related to the sensory characteristics of the dry fermented sausages, which were defined by the trained panel through QDA, as described above.

The consumers were asked to check all of the terms that they considered appropriate to describe each dry fermented sausage. The sensory terms listed was balanced within and across consumers, following *William's Latin Square* experimental design. This implies each consumer received the CATA question with the terms in different order and this order was modified from sample to sampe along the test. This measure is need as primary bias are expected to appear whintin each participant along the test progresses, i.e., there is a frequent seection of terms which more easily catch their attention withing the list of the options (Ares et al., 2014).

Table 1. Descriptors assessed by descriptive quantitative analysis of the dry fermented sausages.

Descriptor	Definition	References
Brightness	Intensity of the reflection of light, observed on a slice of dry fermented sausage at an angle of 45°.	**Little:** jerked beef. **Very:** slice of dry fermented sausage Italian type with five drops of soybean oil uniformly distributed over the surface**.**
Red color	Intensity of the red color observed in the center of the slice of the dry fermented sausage.	**Little:** 1.5 cm slice of salty pork. **Very:** Slice of 1.5 cm dry-cured loins (Perdigão).
Thickness of the board	Measurement of the proportion of darker red color on the edge of the slice of the dry fermented sausage	**None:** 1.0 cm slice of dry fermented sausage Italian type (Sadia). **Very:** 1.0 cm slice of dry fermented sausage Italian type (Nobre).
Acid aroma	Characteristic odor of fermented meat product mainly coming from the lactic acid.	**Little:** dry-cured loins slices, packaged with cover. **Very:** dry fermented sausage Italian type with the addition of 5 drops of water + 1 drop of glacial acetic acid stored in capped container.
Spice aroma	Spicy aroma characteristic of pepper, garlic and nutmeg.	**Little:** 0.5 cm slice of dry fermented sausage Italian type immersed in 50 ml of water for 2 hours. After, a 2 ml aliquot of the solution was sampled and mixed with 10 ml of water. **Very:** 0.5 cm slice of dry fermented sausage Italian type

		immersed in 50 ml of water for 2 hours.
Characteristic aroma of dry fermented sausage	Intensity of the characteristic odor of dry fermented sausage.	**Little:** 0.5 cm slice of dry fermented sausage Italian type immersed in 50 ml of water for 2 hours. After, a 2 ml aliquot of the solution was sampled and mixed with 10 ml of water. **Very:** 0.5 cm slice of dry fermented sausage *Hamburguês* type.
Rancid aroma	Intensity of the characteristic odor of oxidized pork fat	**None:** piece of fresh pork fat. **Very:** 0.5 cm slice of dry fermented sausage *Hamburguês* type (Artisanal) stored for 45 days under refrigeration without vacuum packaging.
Acid taste	Intensity of the characteristic acid taste	**None:** pure water at room temperature. **Very:** 100g of dry fermented sausage Italian type homogenized with 1 ml of glacial acetic acid.
Salty taste	Intensity of salty taste perceived when tasting a slice of dry fermented sausage.	**Little:** 0.20 cm slice of bologna sausage *light*. **Very:** 0.20 cm slice of dry fermented sausage Italian type (Nobre).
Spice flavor	Intensity of the pungent flavor when tasting a slice of dry fermented sausage.	**Little:** 0.20 cm slice of bologna sausage (Perdigão). **Very:** 100g dry fermented sausage Italian type homogenized with 0.80% of white pepper.
Rancid flavor	Intensity of the characteristic flavor of oxidized pork fat	**None:** 0.30 cm slice of turkey sausage *light*. **Very:** 0.20 cm slice of dry fermented sausage Italian type (Frigor Hans).
Residual bitter flavor	Intensity of bitter taste remaining in the mouth after tasting dry fermented sausage.	**None:** 0.20 cm slice of dry fermented sausage Italian type (Seara). **Very:** 100g dry fermented sausage Italian type (Sadia) homogenized with 0.78% KCl + 0.78% $CaCl_2$.
Characteristic flavor of dry fermented sausage	Intensity of the characteristic dry fermented sausage flavor.	**Little:** slice 0.10 cm dry-cured ham. **Very:** 0.20 cm slice of dry fermented sausage Italian type (Sadia).
Chewiness	Time required chewing one slice of dry fermented sausage at a constant rate of force application to reduce it to a consistency suitable for swallowing.	**Little:** slice 0.20 cm dry-cured loins. **Very:** 0.30 cm slice of turkey sausage *light.*
Fattiness	Fat feeling, distributed in the oral cavity.	**Little:** 0.30 cm slice of turkey sausage *light* **Very:** 0.20 cm slice of dry fermented sausage Italian type (Frigor Hans).

2.4 Free listing

One-hundred and six consumers (45% male, 55% female, aged 18-56) were invited to participate in the free listing questionnaire, which was performed one week after the CATA question. The consumers were asked, in a free manner, to write all terms that were related to each of the six samples of dry fermented sausage with respect to the appearance, aroma, flavor, and texture. They were given a sheet of paper with written instructions and asked to complete the task in less than 30 min, on average. The samples were presented in a monadic form and according to balanced complete block design, as reported by MacFie et al. (1989).

2.5 Statistical analysis

One-way analysis of variance (ANOVA), considering samples as the fixed effect (Ares & Deliza, 2010) was applied to the QDA data obtained for each attribute at a 95% significance level. In addition, the principal component analysis (PCA) was also applied to the mean values of attribute intensity using a Person correlation (Aquino et al., 2014).

In the CATA questionnaire, the frequency of use of each term was determined by counting the number of consumers that used that term. The correspondence analysis (CA) considering the chi-square distance (Vidal, Tárrega, Antúnez, Ares & Jaeger, 2015) was also calculated on the matrix containing the frequency of use of each term for each sample. CA is a multivariate method which looks at the correspondence analysis (association) between row and column variables can be regarded in the light of performing a PCA on two nominal (or categorical) variables which make up a two-way contingency table (Silva et al., 2014; Symoneaux, Galmarini & Mehinagic, 2012).

Multidimensional alignment (MDA) was used to evaluate the relationship of the sensory descriptors for each sample. By calculating the value of the cosine of the angle formed between each attribute and sample (range from -1 to 1), it is possible to determine which attributes have strong relationship with each sample and thus complete information about the relationship between products and attributes. Absolute cosines values below 0.707 (=cos (45°) = −cos (135°)) indicate hardly any relationship at all (Carr, Dzuroska, Taylor, Lanza & Pansini, 2009).

For free listing, the total number of descriptors mentioned by all respondents and the minimum, maximum, and average position mentioned by consumers was calculated for each sample. Then, the Smith's salience index (Sj) (equation 1) was calculated for each sensory descriptor in each sample (Libertino et al. 2012). Only the sensory attributes mentioned by at least 10% consumers were included in the data analysis:

$$S_j = \left(\left(\sum_{i=1}^{F_j} (L_i - R_{ij} + 1)/L_i \right) \Big/ N \right)$$

where Sj is the salience index for the attribute j, Fj is the number of respondents who mentioned the attribute j, Li is the length of the list of respondents, Rij is rating given by i interviewed to the attribute j, and N is the total number of respondents. In addition, Principal component analysis (PCA) was performed using the Smith's salience index values obtained for each sample, due the parametric nature of the data.

Hierarchical cluster analysis (HCA) was used to compare the sensory methodologies (QDA, CATA and free listing) through the visual inspection of the dendograms obtained (Matera et al., 2014; Gaze et al. 2015). HCA identifies group of samples with similar sensory characteristics in each methodology, using

input samples' coordinates in the first and second dimension of the sensory maps produced by each technique and considering Manhattan distance, Ward´s agglomeration method, and automatic truncation.

All statistical analyses were performed using XLSTAT software for Windows 2015 (Addinsoft, Paris, France).

3 Results and Discussion

3.1 Quantitative Descriptive Analysis (QDA)

The mean values generated by the QDA for the appearance, aroma, flavor, and texture of the dry fermented sausages are shown in Table 2. The results suggest that the trained panel found differences ($p \leq 0.05$) between all samples for various attributes. This behavior indicates the diversity of the sensory attributes that characterized the dry fermented sausages subjected to the reduction or partial replacement of NaCl.

No differences were observed for the brightness and thickness of the board of the samples subjected to the reduction or replacement of NaCl by KCl and/or $CaCl_2$ compared with the control. Despite the red color of dry fermented sausages being of utmost importance for consumers' acceptance, different values in instrumental color parameters are common in the literature for fermented meat products with a reduction or substitution of NaCl by other chloride salts (Gimeno, Astiasarán & Bello, 1998, 1999, Zanardi, Ghidini, Conter & Ianieri, 2010). The trained panel reported higher scores for the red color of the dry fermented sausages produced with 50% NaCl and 50% KCl (F2) compared with the control ($p < 0.05$).

No significant differences were found for the acid aroma of the dry fermented sausages. The addition of 50% $CaCl_2$ affected the flavor, and significant lower scores were observed for the spicy and characteristic taste of the dry fermented sausages containing 50% $CaCl_2$ (F3). In addition, a higher score was observed for the rancid flavor of these samples. This fact corroborates the reports in the literature on the use of $CaCl_2$ in the formulation of fermented products. The $CaCl_2$ leads to lipid oxidation and thus generates volatile compounds such as hexanal and heptanal (Flores, Nieto, Ferrer & Flores, 2005; Corral, Salvador & Flores, 2013, Dos Santos, Campagnol, Fagundes, Wagner, & Pollonio, 2015). Regarding taste, a lower value was observed in the dry fermented sausages containing only 50% NaCl (F1).

The rancid and bitter taste was more evident in the samples containing 50% $CaCl_2$ (F3), with higher scores compared with the control sample. The change in these attributes led the assessors to reduce the mean scores for the characteristic taste of the samples containing 50% NaCl and 50% $CaCl_2$ (F3). T Lower scores for meat products containing divalent salts may be due to the characteristics of these salts, which can provide residual bitter, astringent, and metallic taste (Flores et al. 2005).

With respect to the texture of the dry fermented sausages, which can be affected by the reduction or replacement of NaCl (Campagnol, Santos, Wagner, Terra & Pollonio, 2011; Dos Santos et al. 2014), the samples containing 50% NaCl and 50% KCl (F2) presented lower values for chewiness compared with the control. No significant difference was observed in fattiness in the dry fermented sausages subjected to reduction or replacement of NaCl, which was expected because the amount of fat added to all of the treatments was 15%.

Table 2. Averages of sensory attributes raised by QDA of dry fermented sausages with 50% reduction of NaCl, and replacement with KCl and / or $CaCl_2$.

Appearance	**Control**	**F1**	**F2**	**F3**	**F4**	**COM**	**MSD***
Brightness	5.5^{bc}	5.9^{b}	5.9^{b}	5.2^{c}	5.7^{bc}	6.8^{a}	1.1
Red color	6.1^{b}	6.4^{ab}	7.1^{a}	6.4^{ab}	6.5^{ab}	5.3^{c}	1.3
Thickness of the board	1.1^{a}	1.2^{a}	1.2^{a}	1.0^{a}	1.0^{a}	0.3^{b}	0.9
Aroma							
Acid	6.4^{a}	6.1^{a}	6.2^{a}	5.7^{a}	6.0^{a}	6.4^{a}	1.5
Spice	6.3^{a}	6.2^{ab}	6.3^{ab}	5.9^{b}	6.0^{ab}	6.6^{a}	1.1
Characteristic	6.5^{ab}	6.4^{b}	6.5^{b}	5.8^{c}	6.3^{bc}	7.1^{a}	1.1
Rancid	0.5^{b}	0.5^{b}	0.7^{b}	2.7^{a}	0.7^{b}	0.2^{b}	1.1
Flavor / Taste							
Acid	6.3^{a}	6.2^{a}	6.0^{a}	5.5^{a}	5.9^{a}	6.0^{a}	1.4
Salty	5.7^{ab}	4.2^{c}	5.7^{ab}	5.6^{ab}	5.4^{ab}	6.0^{a}	1.2
Spice	5.4^{a}	5.1^{a}	5.1^{a}	4.7^{a}	5.1^{a}	4.9^{a}	1.6
Rancid	0.7^{bc}	0.9^{bc}	0.7^{bc}	3.1^{a}	1.1^{b}	0.3^{c}	1.3
Bitter	1.0^{c}	1.4^{c}	2.3^{b}	3.4^{a}	2.3^{b}	0.5^{c}	1.4
Characteristic	6.5^{b}	6.2^{bc}	6.3^{b}	5.5^{c}	6.1^{bc}	7.6^{a}	1.3
Texture							
Chewiness	5.6^{a}	5.7^{a}	4.6^{b}	5.0^{ab}	4.9^{ab}	5.2^{ab}	1.3
Fattiness	5.6^{a}	5.7^{a}	5.9^{a}	5.9^{a}	5.7^{a}	6.2^{a}	1.4

* MSD- Minimum significant difference.

Averages followed by the same letter, the same line did not present significant difference (P≤0.05) by Tukey's test. Control- 100% NaCl, F1- 50% NaCl, F2- 50% NaCl and 50% KCl, F3- 50% NaCl and 50% $CaCl_2$, F4- 50% NaCl, 25% KCl and 25% $CaCl_2$, COM- commercial sample.

The Principal Component Analysis explained 81.57% of the variance of the experimental data in two dimensions (Fig. 1), suggesting that sensory variability involved in the sample set can be represented in only two dimensions. The dry fermented sausages were separated into four main groups according to the combinations of the different salts used as NaCl substitutes and the commercial sample. The first group included the commercial sample (COM), the second group included the control, F1 (50% NaCl), and F2 (50% NaCl and 50% KCl), the third group included the samples containing 50% NaCl and 50% $CaCl_2$ (F3), and the fourth group consisted of dry fermented sausages containing a

blend of chloride salts (F4- 50% NaCl, 25% KCl, and 25% $CaCl_2$). This shows that QDA is a very effective method to discriminate and characterize the reformulated dry fermented sausages and the commercial sample. The commercial sample (COM) was characterized by the attributes of acid, spice, and characteristic aroma, salty taste and characteristic flavor. The control, F1 (50% NaCl) and F2 (50% NaCl and 50% KCl) were characterized by the spice flavor and chewiness. The addition of 50% $CaCl_2$, may have been correlated with the rancid aroma and flavor. However, the reduction of 50% NaCl and the addition of 25% KCl and 25% $CaCl_2$ (F4) showed a correlation with the attributes of red color, thickness of the board, and acid taste.

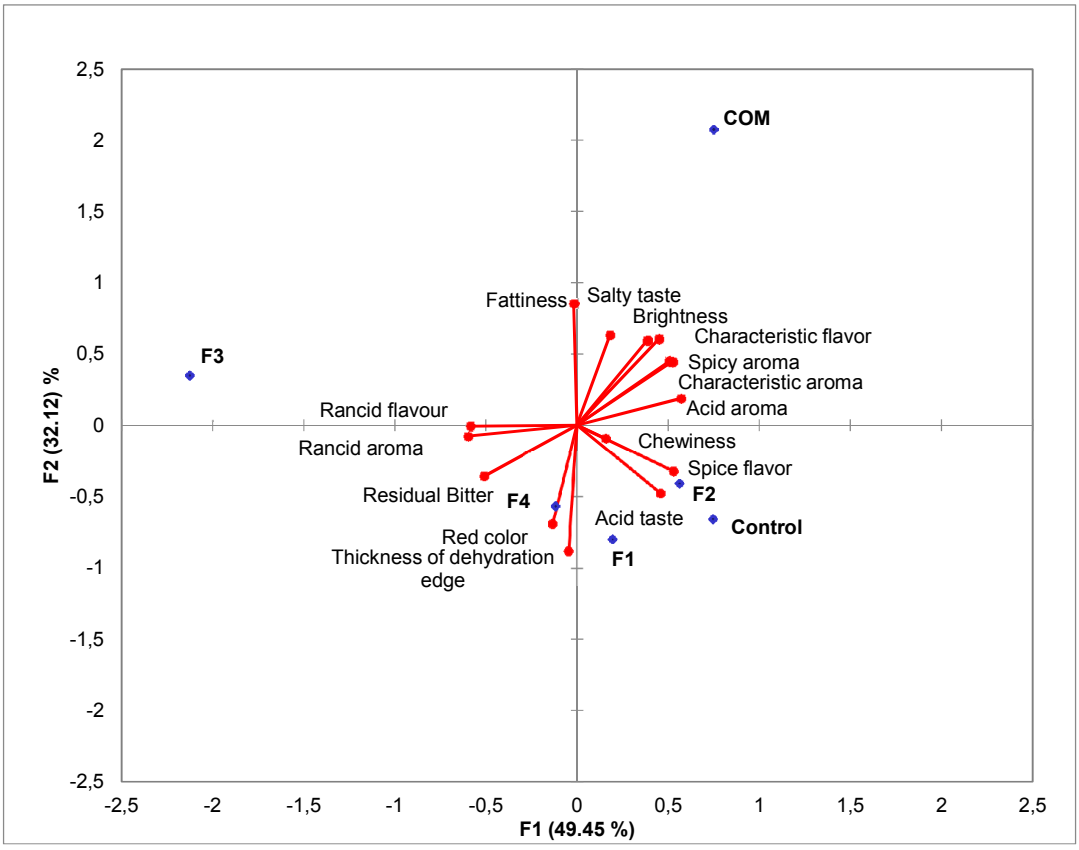


Figure 1. Representation of the samples and the attributes in the first and second dimensions of the principal component analysis performed on the quantitative descriptive analysis (QDA) data. Control- 100% NaCl, F1- 50% NaCl, F2- 50% NaCl and 50% KCl, F3- 50% NaCl and 50% $CaCl_2$, F4- 50% NaCl, 25% KCl and 25% $CaCl_2$, COM- commercial sample.

3.2 Check all that apply

CATA was applied using 15 sensory descriptors, 3 of which described appearance, 4 for aroma, 6 for flavor, and 2 for texture (Table 3). For the control sample, the most common attributes cited were red color (35), characteristic aroma (31) e thickness of the board (28). A greater number of citations of the attributes red color (43), brightness (31), spice aroma (40), acid taste (32), and fattiness (45) were applied to the sample with a 50% NaCl reduction (F1). For the sample with a 50% replacement of NaCl by KCl, the attributes red color (44), brightness (33), spice flavor (31), and fattiness (49) were the most cited by consumers. The samples containing CaCl2 were characterized by the descriptors referring to low sensory quality of reformulated dry fermented sausages. The terms red color (29), bitter taste (29), and fattiness (37) characterized the samples containing 50% $CaCl_2$ (F3), whereas red color (49), brightness (34), acid taste (28) and fattiness (35) characterized the samples produced with the blend of 25% KCl and 25% $CaCl_2$ (F4). A greater number of attributes related to better acceptance was observed for the commercial sample, probably because it was an optimized formulation available for consumers. In addition, the attributes red color (60), brightness (60), characteristic aroma (56), spice aroma (42), characteristic flavor (46), chewiness (46) and fattiness (46) described the commercial sample (COM). Thus, under a food development perspective, these attributes should be taken into account when optimizing low sodium fermented sausages.

Table 3. Frequency mentioned attributes by dry fermented sausage CATA for each sample.

Attributes		Control	F1	F2	F3	F4	COM
Appearance	Red color	35	43	44	29	49	60
	Brightness	22	31	33	21	34	60
	Thickness of the board	28	11	13	16	7	1
Aroma	Acid	22	26	26	23	28	17
	Characteristic	31	28	28	19	26	56
	Rancid	13	10	13	24	10	2
	Spice	28	40	16	20	25	42
Flavor / Taste	Acid	21	32	26	23	28	10
	Salty	23	17	21	11	18	3
	Spice	27	19	31	23	23	13
	Rancid	12	8	17	20	11	15
	Characteristic	27	24	13	14	18	46
	Bitter	4	15	21	29	14	11
Texture	Chewiness	10	7	10	13	18	46
	Fattiness	26	45	49	37	35	46

Control- 100% NaCl, F1- 50% NaCl, F2- 50% NaCl and 50% KCl, F3- 50% NaCl and 50% $CaCl_2$, F4- 50% NaCl, 25% KCl and 25% $CaCl_2$, COM- commercial sample.

The Correspondence Analysis to evaluate the CATA questionnaire explained 81.20% in two dimensions (Fig. 2). The explanation of 81.20% of the total variation of the data characterizes the wealth of information generated by CATA and suggests that this analysis was effective, although this methodology uses consumers who naturally have intrinsic variability. The sensory map suggested that the dry fermented sausages could be separated into three groups. The first group was comprised only of the commercial sample (COM) which was mainly characterized by the sensory attributes of red color, brightness, chewiness, characteristic aroma, and characteristic flavor. Both samples control and F1 (50% NaCl) formed a second group, characterized by the descriptors thickness of the board, spice aroma, and acid taste, and salty taste. A third group formed by the samples subjected to reduction of 50% NaCl and replacement by

chloride salts (F2, F3 and F4) were characterized by many attributes, including rancid flavor, bitter taste, rancid aroma, acid aroma, and fattiness.

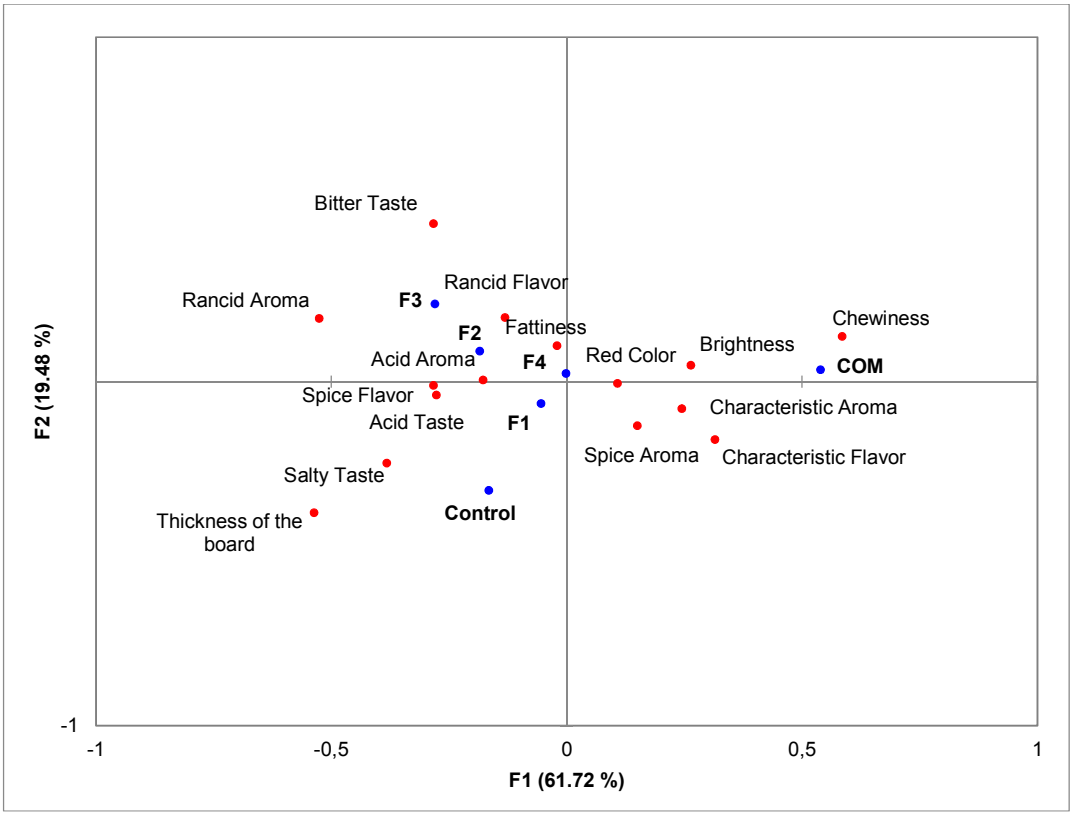


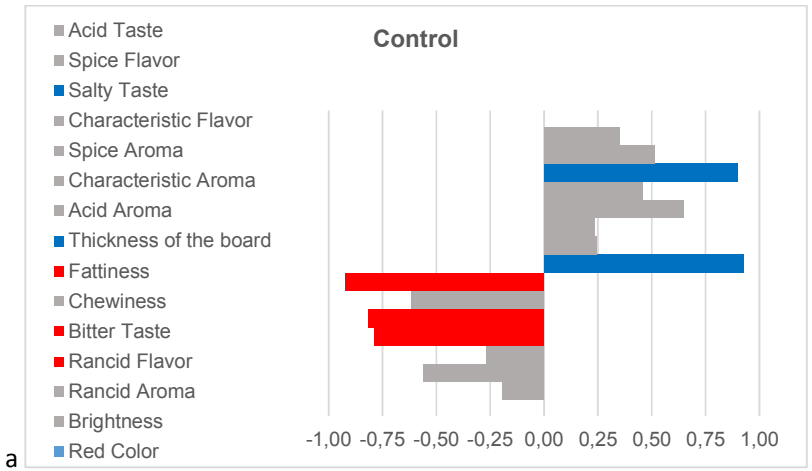
Figure 2. Representation of the samples and the terms in the first and second dimensions of the correspondence analysis performed on check-all-that-apply (CATA) question data. Control- 100% NaCl, F1- 50% NaCl, F2- 50% NaCl and 50% KCl, F3- 50% NaCl and 50% $CaCl_2$, F4- 50% NaCl, 25% KCl and 25% $CaCl_2$, COM- commercial sample.

Multidimensional alignment (MDA) allowed assessing the degree of association between products and attributes on perceptual maps (Carr et al., 2009). Figure 3 (a, b, c, d, e, and f) shows the relationship between the attributes and samples. For interpretation, it should be considered no relationship when the cosine value is below +/- 0.707 (Meyners, Castura & Carr, 2013). According with this criterion, the control sample is associated with the descriptors salty taste, thickness of the board, red color, fattiness, bitter taste and rancid flavor (0.900, 0.927, -0.192, -0,923, -0.814 and -0.789). In turn, the sample with 50% reduction

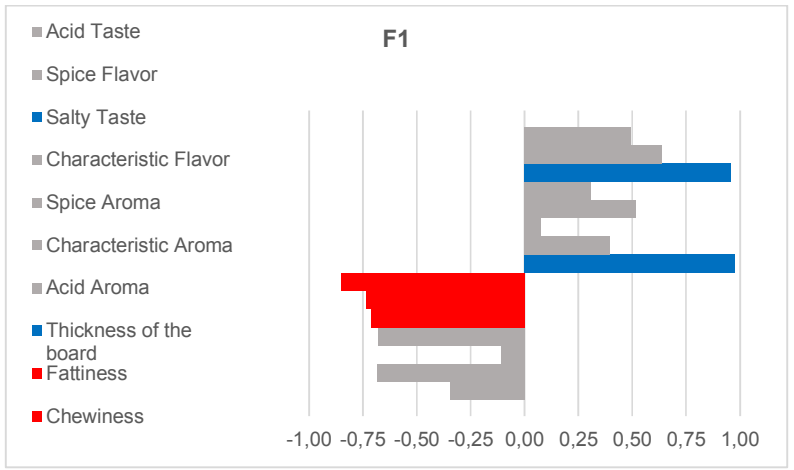
of NaCl (F1) is associated with the descriptors salty taste, thickness of the board, fattiness, chewiness and bitter taste (0.958, 0.975, -0.850, -0.733, and -0.712). The descriptors acid aroma, fattiness, rancid aroma, acid taste, bitter taste, rancid flavor, characteristic flavor, spice aroma, characteristic aroma, and red color (0.786, 0.727, 0.989, 0.712, 0.862, 0.883, -0.999, -0.960, -0.983 and -0.818) were associated with the sample F2 (50% NaCl and 50% KCl). On the other hand, the sample with 50% $CaCl_2$ (F3) is associated with the descriptors fattiness, bitter taste, rancid flavor, rancid aroma, characteristic flavor, characteristic aroma, and spice aroma (0.877, 0.962, 0.973, 0.919, -0.980, -0.904, -1.000). The descriptors fattiness, bitter taste, rancid flavor, salty taste, characteristic flavor, and spice aroma (0.997, 0.954, 0.940, -0.721, -0.709 and -0.851) described the sample containing 25% KCl and 25% $CaCl_2$ (F4). Finally, the commercial sample was characterized for chewiness, characteristic aroma, brightness, red color, acid taste, spice flavor, salty taste, rancid aroma, and acid aroma (0.964, 0.808, 0.980, 0.980, -0.999, -0.990, -0.749, -0.786 and -0.989). It is worth noting that the MDA can correlate the attributes directly to the samples, which is not always available through the two-dimensional map view of the correspondence analysis. One example is the control sample that does not show a clear association with the attributes acid taste and spice flavor when the two-dimensional map of CA is analyzed. This same correlation can be applied to samples F1, F2, F3, F4 and COM, which presented a clear difference between the attributes of the AC and MDA.

The use of CATA suggested some relevant considerations. First, most of the descriptors cited for low sodium samples corresponded to the attribute flavor (above 50% of the total citations of the CATA questionnaire for these samples). This finding supports the importance of this attribute in reduced sodium meat

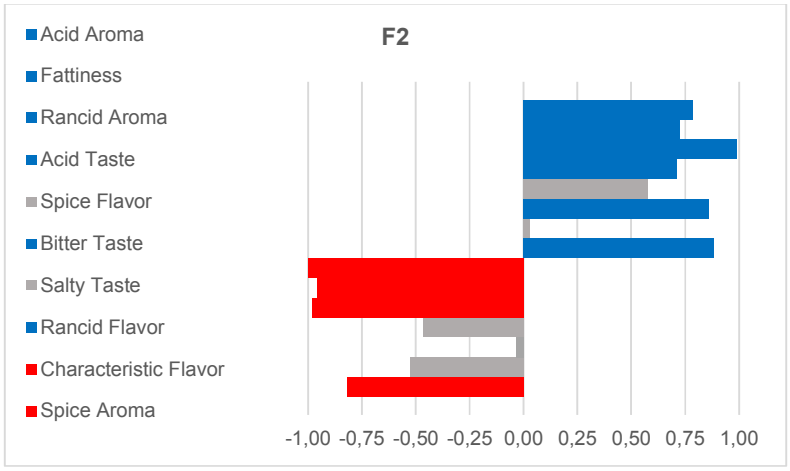
products by decreasing levels of sodium chloride, and in a more global analysis, this is the challenge to be faced by the meat industry.


Control
Acid Taste
Spice Flavor
Salty Taste
Characteristic Flavor
Spice Aroma
Characteristic Aroma
Acid Aroma
Thickness of the board
Fattiness
Chewiness
Bitter Taste
Rancid Flavor
Rancid Aroma
Brightness
Red Color
-1,00 -0,75 -0,50 -0,25 0,00 0,25 0,50 0,75 1,00

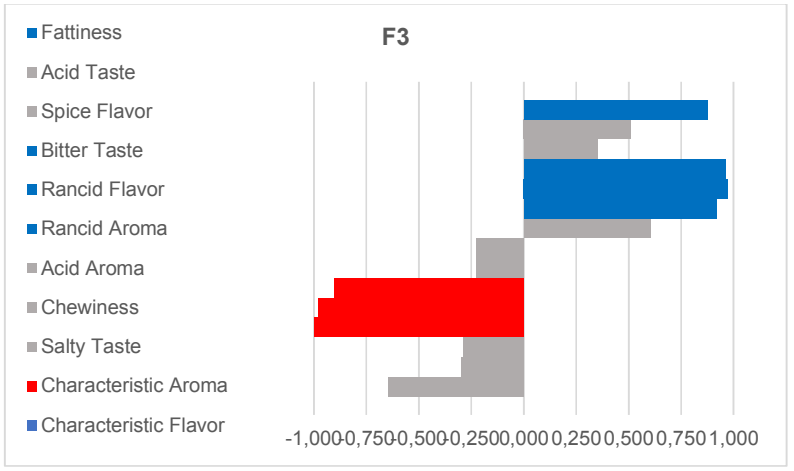
a


F1
Acid Taste
Spice Flavor
Salty Taste
Characteristic Flavor
Spice Aroma
Characteristic Aroma
Acid Aroma
Thickness of the board
Fattiness
Chewiness
-1,00 -0,75 -0,50 -0,25 0,00 0,25 0,50 0,75 1,00

b


F2
Acid Aroma
Fattiness
Rancid Aroma
Acid Taste
Spice Flavor
Bitter Taste
Salty Taste
Rancid Flavor
Characteristic Flavor
Spice Aroma
-1,00 -0,75 -0,50 -0,25 0,00 0,25 0,50 0,75 1,00

c


F3
Fattiness
Acid Taste
Spice Flavor
Bitter Taste
Rancid Flavor
Rancid Aroma
Acid Aroma
Chewiness
Salty Taste
Characteristic Aroma
Characteristic Flavor
-1,000 -0,750 -0,500 -0,250 0,000 0,250 0,500 0,750 1,000

d

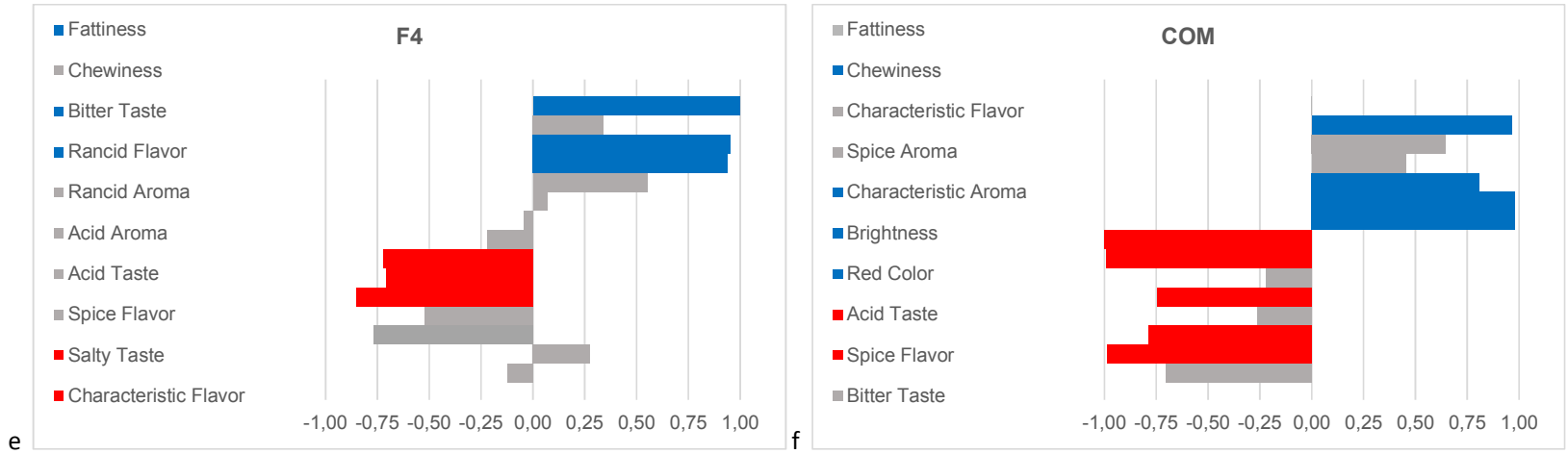


Figure 3. Associations between attributes and samples based on MDA by CATA. Control- 100% NaCl, F1- 50% NaCl, F2- 50% NaCl and 50% KCl, F3- 50% NaCl and 50% $CaCl_2$, F4- 50% NaCl, 25% KCl and 25% $CaCl_2$, COM- commercial sample.

3.3 Free listing

The free listing test generated a list with an average length of 34 items, ranging from 68 to 1. The sample F1 (50% NaCl) showed lists with 10 descriptors, followed by sample COM (commercial) with 7 descriptors, whereas lower lists were observed for the F2 (50% NaCl and 50% KCl) and F4 (50% NaCl, 25% KCl and 25% CaCl2) samples with 2 and 1 descriptors, respectively. Although the time for the execution of the task was not set, a time of 30 minutes was required for participants to perform the task.

The implementation of free listing allowed obtaining a representative vocabulary for the four sensory attributes: appearance, aroma, flavor, and texture. A total of 15 descriptors were developed, 7 of which were related to the attribute flavor (characteristic, fatty, salty, acid, spice, without salt, and bitter) followed by aroma (3 descriptors - acid, characteristic, and rancid), texture (3 descriptors - hard, tender and characteristic), and appearance (2 descriptors, red and pink color). The results emphasized the importance of the attribute flavor in the consumers' perception, evidencing a need for optimization of this attribute during the development of low-sodium meat products.

During the application of the free listing task, the relevance of a term is a cognitive domain defined by two factors: the frequency of mentions and the order of the sensory descriptor in the list provided by the consumers. When analyzed simultaneously, these data provide a more relevant analysis on the importance of each term on the consumer's opinion. About this the most important attributes listed are those cited by a larger number of consumers that rank in the top positions in the list (Libertino et al. 2012). However, the use of these two factors alone provide different information (Ginon, Ares, Issanchou, Laboissière &

Deliza, 2014; Ares et al. 2014); hence, a quantitative parameter is required to express these two factors. Thus, using salience coefficients as the Smith Salience Index used in this study is necessary (Libertino et al. 2012).

Table 4 shows the Smith's Salience Index values (SSI) of the various sensory descriptors of each sample and the frequency of mentions and the average position of these attributes in the free listing task. SSI values exhibited high variation over all of the descriptors that characterize the set of samples. The commercial samples (COM) exhibited the highest SSI values for the descriptor characteristic aroma (0.358), whereas the control (100% NaCl) and F2 (50/50% NaCl / KCl) presented the highest values for the descriptor red color (0.324 and 0.317, respectively). Regarding the number of citations and the average position of the descriptor in the list, the descriptors red color and characteristic aroma generally showed high values regardless of the sample, with values of 49, 37, 39, and 1.30, 1.55, and 1.58, respectively. However, lower values were found for the descriptors characteristic texture (COM) and characteristic flavor (Control), with 0.048 and 12, and 0.111 and 24 citations, respectively. Specific results were observed for the attributes characteristic aroma in the COM sample (34 citations, average position in the list 2.47), and bitter taste (33 citations, average position in the list 2.06) and hard (31 citations, average position in the list 2.42) were observed for the sample with 50% NaCl replaced by KCl (F2). In this context, these descriptors can be more relevant to this product category because they are most frequently listed (Hough & Ferraris, 2010) and should therefore be considered in the development of low-sodium meat products, in particular dry fermented sausages. Interestingly, the descriptor salty taste presented intermediate salient index values, although it is a relevant attribute in the set of

samples under study, ranging from 0.065 (50% NaCl, 25% KCl and 25% CaCl2) to 0.128 (Control), which may be related to a similar perception of this descriptor in all samples, although the control and commercial samples showed 22 and 18 citations, respectively. In fact, the name salty taste and the words as/sodium have been observed in previous studies using a projective map and ultra-flash profile of prebiotic bologna (Santos et al. 2013). In addition, the salty taste has been assigned as a negative sensory descriptor in food, negatively influencing perception (Ares et al. 2014).

Table 4. Indexes calculated for the 15 descriptors terms and total number of respondentes (n=106).

	Control			F1			F2			F3			F4			COM		
	SSI[a]	N[b]	AP[c]	SSI	N	AP	SSI	N	AP	SSI	N	AP	SSI	N	AP	SSI	N	AP
Appearance																		
Red color	0.317	37	1.30	0.243	33	1.55	0.324	39	1.38	0.216	27	1.44	0.039	11	3.00	0.195	25	1.56
Pink color	0.096	12	1.67	0.148	19	1.53	-	-	-	0.190	22	1.23	0.071	16	2.75	0.119	15	1.33
Aroma																		
Ácid	0.185	24	1.67	0.217	29	1.62	0.233	35	1.91	0.191	24	1.42	0.200	28	1.79	0.140	16	1.19
Characteristic	0.265	37	1.68	0.187	29	1.72	0.140	22	1.91	0.154	20	1.55	0.160	21	1.57	0.358	49	1.55
Rancid	-	-	-	-	-	-	-	-	-	0.116	17	1.82	0.078	14	2.14	-	-	-
Flavor/Taste																		
Characteristic	0.111	24	2.29	0.053	16	2.69	0.079	17	2.41	-	-	-	0.068	14	2.14	0.153	34	2.47
Fatty	0.075	12	2.17	0.065	12	2.33	-	-	-	0.100	17	2.18	0.086	14	2.07	0.074	14	1.79
Salty	0.128	22	2.23	0.069	12	2.17	0.105	22	2.50	0.100	17	2.18	0.065	11	2.18	0.077	18	2.50
Acid	0.085	18	2.67	0.115	25	2.64	0.107	20	2.20	0.095	19	2.47	0.057	16	2.88	-	-	-
Spice	0.094	19	2.42	0.102	20	2.35	0.054	15	3.00	-	-	-	-	-	-	-	-	-
Without salt	-	-	-	-	-	-	-	-	-	0.058	12	2.42	0.071	11	1.91	-	-	-
Bitter	-	-	-	-	-	-	0.061	15	2.40	0.205	33	2.06	0.116	22	2.32	-	-	-
Texture	-	-	-															
Tender	0.070	16	2.94	0.093	20	2.70	0.058	12	2.17	0.039	11	3.00	0.063	11	2.27	0.095	24	2.79
Hard	0.090	18	2.56	-	-	-	0.151	31	2.42	0.071	16	2.75	0.072	17	2.47	-	-	-
Characteristic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.048	12	2.83

a- Smith's saliency index; b- number of mentions ; c- average position. Control- 100% NaCl, F1- 50% NaCl, F2- 50% NaCl and 50% KCl, F3- 50% NaCl and 50% $CaCl_2$, F4- 50% NaCl, 25% KCl and 25% $CaCl_2$, COM- commercial sample.

Figure 4 shows the Principal Component Analysis (PCA) of the free listing data, using salience coefficient values. Using two dimensions, it was reported 66.12% of the variance of the experimental data, being 31.76 and 34.36% at the first and second dimension, respectively. The samples Control, F1 (50% NaCl) and F2 (50% NaCl and 50% KCl) were associated to the sensory descriptors red color, spice flavor, salty taste, acid taste, and acid aroma. The samples F3 (50% NaCl and 50% $CaCl_2$) and F4 (50% of NaCl, 25% KCl and 25% of $CaCl_2$) are related to the descriptors fattiness, without salt, rancid aroma, bitter taste and pink color. Finally, the commercial sample (COM) was associated to the descriptors characteristic of dry fermented sausages (aroma, flavor, and texture) besides the descriptor tenderness. The analysis of the two-dimensional map suggests that the groups formed by samples were related to the type of salt substitute used in the formulations. The descriptors for the fermented sausage containing $CaCl_2$ refer to the sensory characteristics that can be developed by this salt, such as bitter and rancid flavor. In contrast the 50% reduction of NaCl and / or 50% addition of KCl provided dry fermented sausages similar to the control, which shows the potential use of KCl as a substitute in these products. This fact was previously reported by dos Santos et al. (2015c), who found a potential consumer market for dry fermented sausages with reduction of 50% NaCl, containing 50% KCl or 25% KCl and 25% $CaCl_2$. In turn, the commercial formulation that is already established and widely consumed, is associated with typical dry fermented sausages attributes, which reflects its familiarity by the consumer. Overall, the results presented by free-listing test seem consistent under the aspects involved in the technology of low sodium fermented sausages which in turn reflect the consumers' perception.

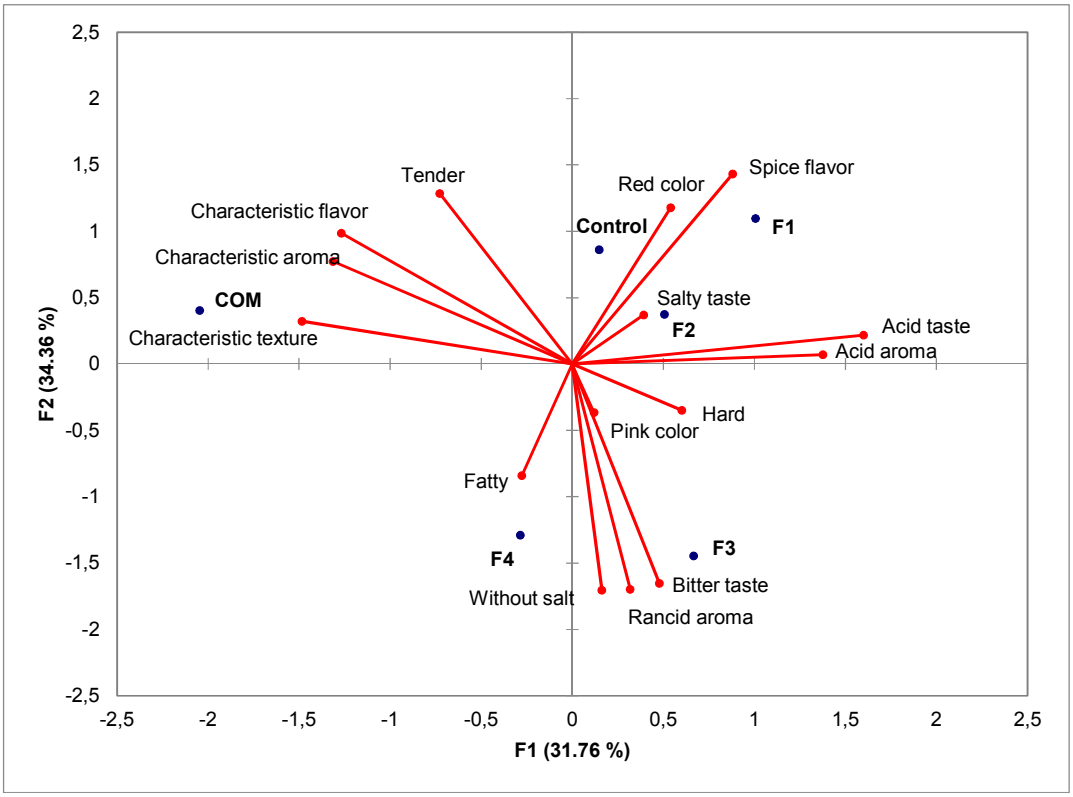


Figure 4. Representation of the samples and the attributes in the first and second dimensions of the principal component analysis performed on the Free listing data. Control- 100% NaCl, F1- 50% NaCl, F2- 50% NaCl and 50% KCl, F3- 50% NaCl and 50% $CaCl_2$, F4- 50% NaCl, 25% KCl and 25% $CaCl_2$, COM- commercial sample.

3.3 Comparison of the sensory methodologies

The use of descriptive sensory methodologies with consumers has the fundamental objective of providing valid and reliable information on sensory characteristics of food products under study. Thus, in addition to verifying whether the methodology reports true and intrinsic sensory attributes corresponding to the set of products, to compare the results with those generated by a panel of assessors trained by quantitative descriptive analysis (QDA) (Ares & Varela, 2014). Although CATA and Free Listing have been widely used in sensory and consumer science, its performance when compared to the same class of

processed products had not yet been assessed, which is the main contribution of this study. Indeed, CATA uses a previous list of defined sensory descriptors while in Free Listing the sensory descriptors are generated spontaneously by consumers, referring to a more natural state of their daily lives. As previously mentioned, when Hough and Ferraris (2010) presented the Free listing method for the scientific community, they have reported the need for evaluation to obtain the sensory descriptors.

The multiple factor analysis (MFA) has been used and the value of the correlation coefficient (Rv) is used without restriction established in this purpose (Cruz et al., 2013, Santos et al., 2013). However, in the free listing test, consumer uses two cognitive strategies, the mention of the descriptor and its respective position in the list created during test application, which is placed in a quantitative manner in calculating the SSI. In turn, CATA and ADQ has only one parameter to be measured, citation frequency of each descriptor, and a note for each descriptor, respectively. In this sense, the MFA is not the most appropriate instrument to compare the methodologies; a good option is the visual comparison in two dimensions through the dendrogram obtained by hierarchical cluster analysis (HCA).

Figure 5 (a, b, c) shows the resulting dendrogram of hierarchical cluster analysis (HCA) obtained by the three methods. Different segments can be viewed according to the method applied, which is related to cognitive strategies used by consumers in the evaluation of the samples in relation to the trained panel. In this sense, all methods demonstrated the existence of three segments. In QDA, we have a group formed by the sample F3 (50% NaCl and 50% $CaCl_2$), commercial sample (COM), and the third group of the samples Control (100%

NaCl), F1 (50% NaCl) F2 (50% NaCl and 50% KCl) and F4 (50% NaCl, 25% KCl and 25% $CaCl_2$), suggesting that the trained panel was able to separate samples of salamis with respect to the type of salt and salt concentrations.

In CATA, three groups were formed by the samples control, commercial, and F1, F2, F3 and F4, respectively. In this case, consumers identified samples with low NaCl content, but were not able to distinguish the KCl or $CaCl_2$ levels. Finally, three groups may also be viewed in free listing: commercial sample (COM) and samples containing 50 and 25% $CaCl_2$ (F3 and F4); sample with 50% KCl (F2) and the third group of the control samples and F1 (50% NaCl). In free listing test, the groups formed have also suggested that consumers perceive the presence of NaCl substitutes, but they were not able to distinguish both the sample containing 50% NaCl and the commercial sample. However, given the less time spent in the execution of the free listing (30 minutes) as compared to QDA (one hour each session), the sample positioning correlation in the HCA between both methodologies evidences the potential use of the free listing as a descriptive methodology to be applied with consumers.

Regarding the vocabulary generated by the different methodologies, the vocabulary generated by free-listing was similar to that developed by the trained panel. This may be related to the methodology performance characteristics, in which the consumer can think freely in the sensory attributes of the product, so with greater attention to describe only the most relevant attributes in its perception. In fact, during the application of CATA, although the task of indicate by check mark the attributes that characterize the product may be easy and present in everyday consumer, there is a great cognitive effort. Factors such as the choice of first attributes (satisfaction strategies) and their order in the list can

result in a test run without the necessary attention (Ares & Jaeger, 2015), which can compromise its efficiency and real characterization of the samples.

In this context, where possible, free-listing test may be interesting to get a more complete, brief, and relevant perception of the sensory attributes of the food product in the consumer's opinion. In case of limitation by human, material, or financial factors, the free listing seems to be the best alternative.

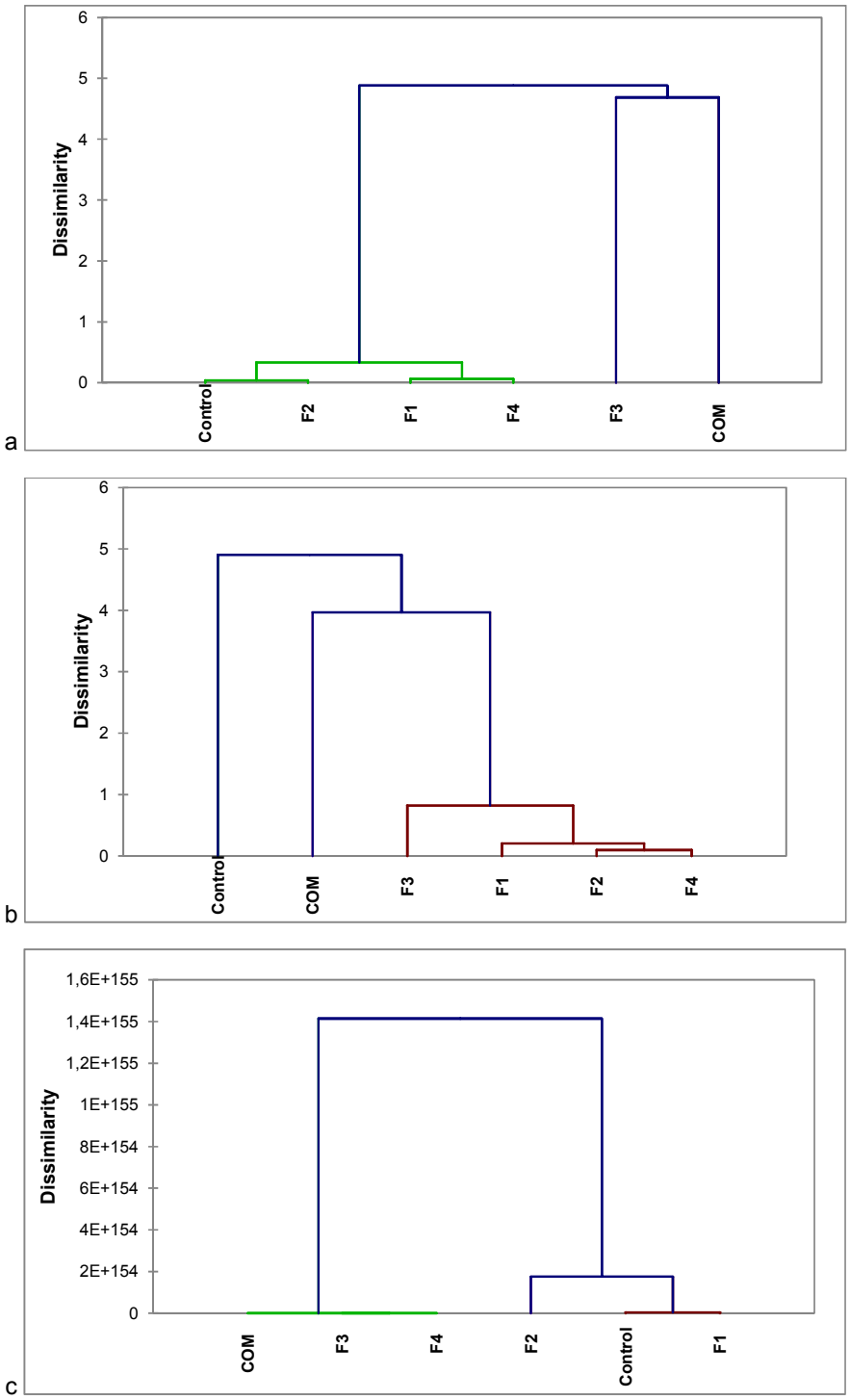


Figure 5. Dendrogram obtained from Hierarchical Cluster analysis (HCA) of QDA (a), CATA (b) and free-listing data (c). Control- 100% NaCl, F1- 50% NaCl, F2- 50% NaCl and 50% KCl, F3- 50% NaCl and 50% $CaCl_2$, F4- 50% NaCl, 25% KCl and 25% $CaCl_2$, COM- commercial sample.

4 Conclusions

Understanding consumers' perceptions is a natural path that can lead to more effective responses of sensory descriptors relevant to optimize the low-sodium meat product formulations. In this context, the use of descriptive sensory methodologies applied to consumers seems to be a valuable alternative due to the economic advantages and the possibility of generating a preliminary vocabulary.

The results of this study suggests the CATA questionnaire and free-listing do not present a similar configuration when compared with the panel trained by QDA, which is related to different cognitive strategies of the methodologies. However, free listing, in particular, was able to generate an additional and complementary vocabulary to that obtained in QDA and more detailed for other sensory descriptors.

Free listing presented as an interesting, useful, and potential alternative to be performed with consumers aimed to assess the sensory characteristics of this product category, with the advantage of absence of a list of attributes previously defined by a trained panel. Our findings may contribute to the use of free listing in reformulation studies on the sensory profiles of processed products, in particular, fermented meat products. Future studies are needed to confirm this hypothesis.

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CONCLUSÃO GERAL

Conclusão Geral

Os resultados do estudo indicaram que a simples redução de 50% de NaCl não ocasionou alterações negativas no processo de fabricação dos salames, mas interferiu no desenvolvimento das reações proteolíticas e lipolíticas ao diminuir a intensidade da proteólise e geração de compostos voláteis provenientes da oxidação lipídica.

Para os salames produzidos com 50% de NaCl e 50% de KCl as propriedades físico-químicas (pH, Aw e perda de peso), microbiológicas (contagem total, bactérias láticas e micrococáceas) e reações proteolíticas mantiveram-se sem alteração durante o processamento. Uma diminuição na firmeza dos salames foi observada durante o armazenamento. Os compostos voláteis provenientes da degradação de aminoácidos e fermentação de carboidratos foi maior nestes salames durante o armazenamento o que pode ter contribuído de forma positiva para a qualidade sensorial dos produtos. Um grupo de consumidores consideraram os salames com 50% de KCl com boa aceitação sensorial através do estudo de clusters.

A presença de $CaCl_2$ nos salames elaborados com 50% de NaCl e 50% de $CaCl_2$ e pelo *blend* de 25% de KCl e 25% de $CaCl_2$ provocou alterações negativas no processo de fabricação dos salames, como aumento da atividade de água, redução do pH e menor contagem de bactérias láticas e micrococáceas. Os parâmetros de cor L* e *whiteness* tiveram um decréscimo e a* e b* um aumento durante o armazenamento. Além disso, foi observada uma modificação no perfil de eletroforese das proteínas sarcoplasmáticas, aumento de aminoácidos livres, aumento da dureza durante o armazenamento, alto valor de TBARs, degradação de ácidos graxos poli-insaturados e um aumento de compostos voláteis provenientes da oxidação lipídica. Os salames adicionados

de 50% de $CaCl_2$ tiveram uma baixa aceitação sensorial e foram caracterizados pelo sabor e aroma de ranço. Este comportamento mostra que o $CaCl_2$ não é melhor alternativa para reduzir o teor de NaCl em salames.

Do ponto de vista sensorial, os testes ADQ, CATA e Free Listing foram complementares entre si e foram capazes de caracterizar sensorialmente os salames com reduzido teor de NaCl. Além disso, os salames do estudo tiveram uma redução média de 42% de sódio e um aumento de potássio e cálcio que contribuíram para a qualidade nutricional dos produtos reformulados. Dessa forma, os resultados do estudo indicaram que do ponto de vista tecnológico, microbiológico e sensorial os salames produzidos com 50% de NaCl e 50% de KCl é a formulação mais indicada como alternativa para a redução de sódio em produtos cárneos fermentados.

ANEXO

FACULDADE DE CIENCIAS MEDICAS - UNICAMP (CAMPUS CAMPINAS)

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Redução de cloreto de sódio em salames: efeito de sais substitutos (cloreto de potássio e cloreto de cálcio) sobre as reações bioquímicas e sensoriais.

Pesquisador: BIBIANA ALVES DOS SANTOS

Área Temática:

Versão: 1

CAAE: 07862712.6.0000.5404

Instituição Proponente: Faculdade de Engenharia de Alimentos

DADOS DO PARECER

Número do Parecer: 130.260

Data da Relatoria: 25/09/2012

Apresentação do Projeto:

Com a execução do projeto, espera-se reunir informações científicas consistentes para recomendar estratégias tecnológicas seguras e viáveis para reduzir teores de sódio em produtos cárneos fermentados, como salame. A descrição e interpretação dos mecanismos bioquímicos que ocorrem quando se reduz cloreto de sódio em produtos cárneos fermentados servirá de guia para novas propostas de reformulação, aplicação de novos ingredientes e até alterações de programas de fermentação e secagem, uma vez que as etapas críticas serão destacadas

Objetivo da Pesquisa:

Objetivo Primário:

O objetivo geral do presente trabalho é avaliar o efeito da redução parcial de NaCl e substituição por blends de cloreto de potássio e cloreto de cálcio

sobre as reações bioquímicas e sensoriais de salames durante as etapas de fabricação.

Objetivo Secundário:

Os objetivos específicos serão:

- Avaliar os produtos da degradação proteica através de estudos de proteólise em salames com redução de cloreto de sódio.

- Analisar os produtos resultantes da atividade lipídica (lipólise e oxidação lipídica) em diferentes tempos de maturação de salames com redução de sódio.

- Caracterizar um embutido fermentado com redução de sódio, através de uma análise descritiva quantitativa (ADQ).

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- Correlacionar os resultados obtidos no perfil sensorial com as reações de lipólise e oxidação lipídica dos salames.

Avaliação dos Riscos e Benefícios:

Não haverá benefícios diretos aos sujeitos de pesquisa, porém a participação deverá ajudar no desenvolvimento de produtos com menor teor de sódio, portanto melhores a saúde. Não há riscos previsíveis na pesquisa.

Comentários e Considerações sobre a Pesquisa:

Tese de doutorado da FEA. Projeto bem elaborado e com objetivos bem definidos. Estudo do tipo análise sensorial.

Considerações sobre os Termos de apresentação obrigatória:

todos os termos estão em ordem.

Recomendações:

sem recomendações

Conclusões ou Pendências e Lista de Inadequações:

sem pendências.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

CAMPINAS, 24 de Outubro de 2012

Assinador por: Carlos Eduardo Steiner (Coordenador)