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DE ALIMENTOS**

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**ELABORAÇÃO DE PERNIL CURADO DE COELHO COM REDUZIDO  
TEOR DE SÓDIO**

**Santa Maria, RS  
2021**

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SÓDIO**

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, Área de Concentração em Ciência e Tecnologia dos Alimentos da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutor em Ciência e Tecnologia dos Alimentos**.

**Orientador: Profº. Dr. Paulo Cesar Bastianello Campagnol**

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**Douglas Pedro**

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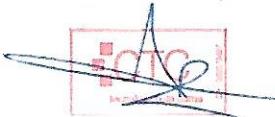
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## RESUMO

### ELABORAÇÃO DE PERNIL CURADO DE COELHO COM REDUZIDO TEOR DE SÓDIO

**AUTOR:** Douglas Pedro  
**ORIENTADOR:** Paulo Cezar Bastianello Campagnol

Produtos cárneos curados normalmente são produzidos com teores elevados de sal para garantir a qualidade microbiológica do produto, além de desenvolver características sensoriais desejáveis. O objetivo deste estudo foi elaborar pernil curado de coelho com reduzido teor de sódio e avaliar o efeito desta reformulação sobre suas características físicas, químicas e sensoriais. Os pernis foram elaborados com redução ou substituição de 50% do NaCl por KCl e com adição de Glutamato Monossódico (GM). Após a salga, os pernis foram colocados em uma câmara de maturação que foi regulada nas seguintes condições de temperatura e umidade relativa (UR): dias 1 a 7:  $2 \pm 1^\circ\text{C}$  e  $90 \pm 5\%$  UR; dias 8 a 28:  $12 \pm 1^\circ\text{C}$  e  $70 \pm 5\%$  UR. A reformulação sódica não causou grande impacto na Aw, pH e perda de peso das amostras durante o processamento. Todos os tratamentos apresentaram altos teores de proteína ( $> 30\%$ ) e baixos teores de gordura ( $< 6,0\%$ ). Todos os aminoácidos essenciais apresentaram um escore de aminoácidos acima de 1,0. O ácido oleico foi o principal ácido graxo (AG), representando quase 1/3 do total. Todos os tratamentos reformulados tiveram uma redução de sódio próxima a 45% e as amostras com adição de KCl apresentaram uma relação saudável de Na/K. Uma menor salinidade foi percebida pelos consumidores no teste *sorting task* nas amostras com redução de 50% de NaCl. As amostras com KCl apresentaram no teste de aceitação menores notas ( $P < 0,05$ ) nos atributos de sabor e aceitação global e foram caracterizadas no teste CATA pelos atributos “sabor adstringente”, “sabor amargo” e “sabor metálico”. O GM conseguiu aumentar a salinidade das amostras com 50% de redução de NaCl, porém, não foi eficiente para compensar todos os defeitos sensoriais causados pelo KCl. A evolução dos valores de TBARS, pH e potencial redox durante 90 dias de armazenamento a  $20^\circ\text{C}$  demonstrou que a reformulação sódica não afetou a estabilidade oxidativa do produto.

**Palavras chave:** coelho, redução de sódio, KCl, realçador de sabor, carne exótica, *sorting task*, CATA.

## **ABSTRACT**

### **MANUFACTURE OF LOW-SODIUM DRY-CURED RABBIT LEG**

**AUTHOR:** Douglas Pedro  
**ADVISOR:** Paulo Cezar Bastianello Campagnol

Cured meat products are usually produced with high salt levels to guarantee the microbiological quality of the final product and to improve the sensory characteristics. This study aimed to produce low-sodium dry-cured rabbit leg, and to evaluate the effect of this reformulation on the physicochemical and sensory characteristics. The legs were made with a reduction or replacement of 50% of NaCl by KCl and the addition of monosodium glutamate (MG). After salting, the legs were placed in a ripening chamber under the following temperature and relative humidity (RH) conditions:  $2 \pm 1$  °C and  $90 \pm 5\%$  RH from day 1 to 7; and  $12 \pm 1$  °C and  $70 \pm 5\%$  RH from day 8 to 28 of storage. The sodium reformulation had no major impact on aw, pH, and weight loss of the samples during processing. All treatments showed high protein ( $> 30\%$ ) and low fat ( $< 6.0\%$ ) levels. The amino acid score was above 1.0 for all essential amino acids. Oleic acid was the main fatty acid (FA), representing almost 1/3 of the total fatty acids. All reformulated treatments presented a sodium reduction close to 45%, and the formulations made with the addition of KCl showed a healthy Na/K ratio. A lower saltiness perception was reported by consumers in the sorting task for the samples with a 50% salt reduction. The formulations containing KCl showed lower flavor and overall acceptance scores ( $P < 0.05$ ), and were characterized in the CATA test by the attributes "astringent flavor", "bitter taste" and "metallic taste". Although MG led to an increase in the saltiness of the formulations made with a 50% salt reduction, it was not effective to suppress all the sensory defects caused by the addition of KCl. The changes observed in TBARS values, pH, and redox potential throughout 90 days of storage at 20 °C demonstrated that the sodium reformulation did not affect the oxidative stability of the product.

**Keywords:** rabbit, sodium reduction, KCl, flavor enhancer, exotic meat, sorting task, CATA.

## **LISTA DE ABREVIATURAS E SIGLAS**

|          |  |
|----------|--|
| AI       | Índice de aterogenicidade                                    |
| COVID-19 | Doença por Coronavírus 2019                                  |
| Eh       | Potencial Redox  |
| FAO      | Organização das Nações Unidas para Agricultura e Alimentação |
| GM       | Glutamato Monossódico  |
| OMS      | Organização Mundial de Saúde                                 |
| ONU      | Universidade das Nações Unidas                               |
| pH       | Potencial Hidrogênionico                                     |
| PUFA     | Ácidos Graxos Poliinsaturados                                |
| SFA      | Ácidos Graxos Saturados                                      |
| TBARS    | Substâncias reativas ao ácido tiobarbitúrico                 |
| TI       | Índice de trombogenicidade                                   |
| WHO      | Organização Mundial de Saúde                                 |
| CATA     | Teste sensorial “CHECK ALL THAT APPLY“                       |

## SUMÁRIO

|  |    |
|--|----|
| <b>1 INTRODUÇÃO .....</b>  | 9  |
| <b>2 OBJETIVOS .....</b>   | 11 |
| 2.1 Objetivo geral .....   | 11 |
| 2.2 Objetivos específicos.....   | 11 |
| <b>3 REVISÃO BIBLIOGRAFICA .....</b>   | 12 |
| 3.1 Produção e consumo de carne de coelho .....  | 12 |
| 3.2 Cadeia produtiva da cunicultura brasileira .....   | 13 |
| 3.3 Qualidade nutricional da carne de coelho e sua utilização para elaboração de produtos mais saudáveis.....              | 13 |
| <b>4 ARTIGOS CIENTÍFICOS .....</b>   | 16 |
| 4.1 Artigo 1: Low-sodium dry-cured rabbit leg: a novel meat product with healthier properties.....                         | 16 |
| 4.2 Artigo 2: Sodium reformulation and its impact on oxidative stability and sensory quality of dry-cured rabbit legs..... | 27 |
| <b>5 DISCUSSÃO GERAL .....</b>   | 51 |
| <b>6 CONCLUSÃO GERAL .....</b>   | 54 |
| <b>REFERÊNCIAS .....</b>   | 56 |

## 1 INTRODUÇÃO

A pandemia de COVID-19 modificou o estilo de vida de muitos consumidores e a busca por uma alimentação capaz de fortalecer o sistema imunológico está cada vez mais em evidência (GALANAKIS, 2020). Desta forma, a demanda por alimentos com características mais saudáveis deve aumentar nos próximos anos. A indústria cárnea pode participar deste nicho de mercado, mas para que isto aconteça é necessário inovar e melhorar a qualidade nutricional de seus produtos.

A carne de coelho por si só possui uma excelente qualidade nutricional, pois é rica em ácido oleico, proteínas e aminoácidos essenciais e tem baixo teor de gordura e colesterol (LI et al., 2018). Somado a isso, a carne de coelho tem pouca alergenicidade e possui quantidades significativas de minerais como selênio e fósforo, bem como de vitaminas do complexo B (CULLERE et al., 2018; 2019).

No entanto, existe uma série de problemas que fazem com que não ocorra um desenvolvimento da cadeia produtiva da carne de coelho, tais como a ausência de indústrias especializadas, poucos investimentos na área e número de pesquisas abaixo do necessário (LI et al., 2018). Além disso, críticas por parte dos consumidores ligadas ao bem-estar dos animais durante o seu período de criação (MATICS et al., 2014) e um consumo cada vez menor da carne de coelho, tem se tornado uma grave ameaça ao futuro desse mercado (PETRACCI et al., 2018).

Por esses motivos, novas estratégias são necessárias com urgência para estimular o consumo dessa carne de alto valor nutricional e garantir a sobrevivência dessa indústria. Atualmente existe uma pequena variedade de produtos elaborados com carne de coelho. Desta forma, a utilização desta matéria-prima para a elaboração de novos produtos cárneos mais saudáveis pode ser uma estratégia eficiente para atrair novos consumidores, especialmente aqueles preocupados com a saúde. Somado a isso, o processamento pode mascarar o típico sabor de carne de caça que a carne de coelho possui, o que pode resultar em rejeição de compra por novos consumidores (CULLERE & ZOTTE, 2018).

O presunto cru é um produto nobre da indústria cárnea que é elaborado tradicionalmente com o pernil suíno e que permite uma significativa agregação de valor a matéria-prima. Esse produto é elaborado aliando tradição com a mais alta tecnologia e é muito apreciado em todo o mundo por sua qualidade sensorial e nutricional. Uma das grandes vantagens do presunto cru é a sua baixa atividade de água, o que permite que seja comercializado a temperatura ambiente dispensando a cadeia do frio (PRETOVA et al., 2016). Além disso, pesquisas comprovaram que durante a etapa de maturação são gerados peptídeos bioativos que

desempenham diversas funções benéficas no organismo humano (TOLDRÁ et al., 2020). Outra grande vantagem é que este produto tem um apelo de rótulo limpo, pois na maioria dos países, geralmente é elaborado com apenas NaCl, nitrito e nitrato (GALLEGÓ et al., 2018; ESCUDERO et al., 2013).

No entanto, por apresentar um teor de NaCl superior a 5% (ARMENTEROS et al., 2012a), o consumo frequente e em excesso de presunto cru pode contribuir para aumentar os fatores de risco relacionados ao surgimento de doenças cardiovasculares (RICO-CAMPÀ et al., 2020), as quais são as principais causas de morte e invalidez de pessoas em todo o mundo atualmente (BENNETT et al., 2018). Além disso, com um cenário atual de pandemia tornou-se imprescindível uma alimentação balanceada, pois com a necessidade de se ficar mais tempo em casa surgiram hábitos alimentares pouco saudáveis (MASON et al., 2021). Desta forma, a redução no teor de sódio dos produtos cárneos pode reduzir o desenvolvimento da hipertensão arterial e consequentemente o risco de morte por COVID-19 (HE et al., 2020; FANG et al., 2020).

A substituição de NaCl por KCl é uma das estratégias mais factível para reduzir o teor de sódio de produtos cárneos (PATEIRO et al., 2021). Várias estratégias já foram pesquisadas para reduzir o teor de sódio de presunto cru (INGUGLIA et al., 2017). A substituição de NaCl por KCl é uma das abordagens que apresenta melhores resultados (PETIT et al., 2019; ARMENTEROS et al., 2012b). No entanto, o KCl também pode afetar a estabilidade oxidativa durante a vida útil dependendo do nível que é utilizado e do tipo de produto cárneo (DOS SANTOS et al., 2017; VIDAL et al., 2019). Além disso, é bem documentado que o KCl pode conferir um sabor amargo e metálico e uma sensação adstringente quando utilizado em níveis a partir de 50% de substituição de NaCl (SANTOS ALVES et al., 2017; DA SILVA et al., 2020). De acordo com Inguglia et al. (2017), a adição de realçadores de sabor é uma estratégia que deve ser explorada para reduzir os defeitos sensoriais do KCl. Neste sentido, em um estudo anterior nosso grupo de pesquisa demonstrou que a utilização de realçadores de sabor, como o Glutamato Monossódico (GM), foi eficiente para reduzir os defeitos sensoriais causados pela adição de altos níveis de KCl em embutidos fermentados (DOS SANTOS et al., 2014).

Até o momento, a utilização da carne de coelho para a elaboração de produtos curados e conservados a temperatura ambiente e que tenham um apelo mais saudável tem sido pouco explorada. Desta forma, neste estudo foi avaliado o efeito da redução ou da substituição de 50% de NaCl por KCl e da adição de GM na qualidade tecnológica, nutricional, oxidativa e sensorial de pernis curados de coelho.

## **2 OBJETIVOS**

### **2.1 Objetivo geral**

- Produzir pernil curado de coelho com reduzido teor de sódio através da redução ou da substituição de 50% de NaCl por KCl e da adição de GM.

### **2.2 Objetivos específicos**

- Avaliar o efeito da reformulação sódica na evolução do pH, atividade de água (Aw) e perda de peso durante o processamento dos pernis curados.
- Ao término da fase de elaboração, avaliar a composição química do produto, a produção de compostos voláteis, ácidos graxos e posteriormente submeter o produto à avaliação sensorial.
- Determinar a qualidade oxidativa dos produtos durante o armazenamento (90 dias a 20°C em ausência de luz).

### **3 REVISÃO BIBLIOGRAFICA**

#### **3.1 Produção e consumo de carne de coelho**

O consumidor está mais atendo ao que consome e busca alimentos que possam lhe proporcionar maior saúde (ZOTTE & SZENDRÓ, 2011). A carne de coelho é a quinta carne de maior consumo no mundo (ESCRIBA-PEREZ et al., 2017), sendo muito popular na China que atualmente é o maior produtor mundial. Diversas pesquisas sobre o processamento da carne de coelho têm sido conduzidas pelo mundo, porém a forma mais comum de comercialização continua sendo em cortes in natura ou mesmo carcaças inteiras (LI et al., 2018; PETRACCI & CAVANI, 2013).

A Europa é o segundo maior produtor mundial de carne de coelho, porém, atualmente encontra-se numa redução progressiva no consumo. Um grande número de críticas relacionadas com o bem-estar dos animais e outras questões éticas acabam tornando o futuro dessa indústria incerto e possivelmente com chances de perda dos conhecimentos técnicos da área adquiridos ao longo dos anos (CULLERE & ZOTTE, 2018).

De acordo com Napolitano et al. (2010) houveram mudanças no estilo de vida das pessoas e nas relações dos consumidores com os alimentos e somado a isso, um aumento com a preocupação na forma como os animais são criados e com os padrões de bem-estar animal. Sendo este determinante para o consumidor na hora de escolher a carne e quando se trata de carne de coelho essa atitude é ainda mais forte, pois progressivamente esse animal passa a ser mais considerado como um animal de estimação do que de consumo (CULLERE & ZOTTE, 2018).

No Brasil o consumo da carne de coelho é de aproximadamente 200g por habitante ao ano, ou seja, muito pequeno quando comparado ao europeu que chega a 7kg. Num trabalho conduzido no interior do Rio Grande do Sul, observou-se que a falta de conhecimento por parte dos consumidores das qualidades nutricionais dessa carne é um fator importante e limitante no consumo (VELASQUES et al., 2020).

Dessa maneira, é necessário que se busquem formas de estimular as pessoas a consumirem essa carne, de maneira que com o desenvolvimento de novos produtos ela se torne mais atrativa e conveniente. É através da inovação com o processamento dessa carne e o desenvolvimento de novos produtos, que a carne de coelho poderá ter um espaço mais relevante no mercado de alimentos (PETRACCI et al., 2018).

### **3.2 Cadeia produtiva da cunicultura brasileira**

No Brasil a maior parte dos cunicultores desenvolve a atividade criatória de maneira secundária. Os números mostram que a maioria dos animais estão alojados em propriedades de pequeno porte com no máximo 10 hectares (MACHADO & FERREIRA, 2014).

De uma maneira geral a cadeia produtiva está desorganizada, existindo apenas algumas iniciativas isoladas com alguns produtores e poucas fábricas de ração e indústrias de beneficiamentos de subprodutos. A maioria dos produtores de carne cúnica estão localizados nos estados do sul do país, sendo a maior parte no Rio Grande do Sul, onde existem modelos de integração muito semelhantes aos adotados na cadeia de aves de corte (MACHADO, 2012).

A quantidade insuficiente de pesquisas na área, somada a ausência de incentivos governamentais, acabam sendo limitantes ao desenvolvimento da produção de coelhos no Brasil. Porém a grande prolificidade da espécie, o baixo potencial poluidor e o fácil manejo, somados a excelente qualidade da carne, projetam um crescimento do setor (FERREIRA et al., 2012). Além disso, a carne de coelho vem se tornando mais competitiva em termos de preço, principalmente pela melhora genética, técnicas de criação melhoradas e diminuições nos custos de produção (KAC, 2015).

Porém, ainda existem diversos entraves para o consumo da carne de coelho no Brasil, os quais na grande maioria estão diretamente ligados aos hábitos de consumo do brasileiro, além da falta de conhecimento da maioria dos consumidores quanto aos benefícios nutricionais da carne de coelho (BONAMIGO et al., 2017).

### **3.3 Qualidade nutricional da carne de coelho e sua utilização para elaboração de produtos mais saudáveis**

A carne de coelho quando comparada as demais, apresenta um alto teor de proteínas, menor teor de colesterol e grande digestibilidade. É uma fonte importante de vitaminas do complexo B, com altos níveis de fósforo e baixos níveis de sódio o que favorece seu consumo por pessoas hipertensas. Essas características ainda podem ser melhoradas através do enriquecimento das dietas dos coelhos com compostos bioativos, como óleo de linhaça ou mesmo óleo de peixe, tornando a carne desse animal um produto funcional, atendendo um anseio crescente dos consumidores preocupados com a relação de saúde e dieta (ZOTTE, & SZENDRO, 2011).

Mesmo com tantas características desejáveis do ponto de vista de saúde, o aspecto visual acaba causando grande impacto no consumo e dessa forma pesquisas com o intuito de melhorar a imagem da carne de coelho, tanto para os consumidores habituais como para aqueles que nunca consumiram é um passo importante para aumentar o consumo (CULERE & ZOTTE, 2018).

Segundo Petracci & Cavani (2013), mesmo com o processamento da carne de coelho com o intuito de torná-la mais atrativa, a maior parte ainda é comercializada na forma in natura. O alto custo de produção quando comparada com outros tipos de carnes é um fator que limita drasticamente seu uso na criação de novos produtos. Além disso, o processamento da carne de coelho se torna um desafio devido a existência de alguns fatores como a baixa suculência (ZOTTE, 2002).

Na China existem alguns produtos processados oriundos da carne de coelho, porém a maioria desses produtos são elaborados de forma artesanal o que torna difícil a produção em grande escala (LI et al., 2018). Segundo CULERE & ZOTTE (2018) a criação de novos produtos é uma das estratégias para que a indústria da carne de coelho garanta sua sobrevivência mantendo os consumidores tradicionais e podendo atrair novos adeptos ao consumo dessa carne. No entanto, o grande desafio dos profissionais envolvidos com essa cadeia produtiva é baixar os custos de produção, pois devido ao tamanho reduzido dessa indústria, a pouca automação e o baixo desenvolvimento tecnológico, os custos ainda são elevados (PETRACCI & CAVANI, 2013).

Na China estão disponíveis diversos produtos processados com carne de coelho como defumados, assados, enlatados, curados, secos, conservados em molho e salsicha que auxiliam fortemente no desenvolvimento da indústria local, porém há uma escassez de produtos que atendam de forma conveniente o consumidor (LI et al., 2018).

O processamento excessivo pode reduzir e prejudicar a qualidade da carne, desta forma os aditivos alimentares têm sido usados com o intuito de melhorar os atributos sensoriais e a aceitação dos produtos pelos consumidores (LI et al., 2018).

Um ingrediente importante no processamento é o sal, sendo essencial na elaboração de produtos cárneos (PETIT et al., 2019). O sal participa no auxílio do controle microbiano, está envolvido no desenvolvimento da textura e do sabor, facilita a elaboração de emulsões e aumenta o rendimento dos produtos (INGUGLIA et al., 2017; DESMOND, 2006). Porém, o consumo em excesso de sal pode levar ao desenvolvimento de doenças ligadas ao sistema cardiovascular, renal, obesidade, câncer no estômago e também por complicações potencializadas pelo COVID-19, inclusive a morte (HE et al., 2020; FANG et al., 2020).

O KCl é o substituto do NaCl mais utilizado em produtos cárneos curados com o intuito de reduzir os níveis de sódio (WU et al., 2015) pois ele possui um efeito antimicrobiano muito similar ao NaCl (BIDLAS, & LAMBERT, 2008). Pesquisas mostraram que o uso de GM vem sendo uma boa alternativa para compensar a redução de salinidade (HE et al., 2011) e pode ser uma boa estratégia para aumentar a sensação do sabor salgado nos produtos com redução nos níveis de sódio (PEDRO et al., 2021). Em um estudo recente nosso grupo de pesquisa utilizou a carne de coelho para elaborar um produto cárneo maturado com características mais saudáveis. A estratégia utilizada foi produzir um pernil curado de coelho com redução ou substituição de 50% de NaCl por KCl e adição de glutamato monossódico (GM) como realçador de sabor. Foi aplicado o teste “sorting task” para explorar a percepção sensorial dos consumidores deste novo produto e os resultados sugeriram que o KCl reduziu a salinidade e que o GM reduziu os defeitos sensoriais causados pelo KCl (PEDRO et al., 2021).

## 4 ARTIGOS CIENTÍFICOS

### **4.1 Artigo 1: Low-sodium dry-cured rabbit leg: a novel meat product with healthier properties**

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## Low-sodium dry-cured rabbit leg: A novel meat product with healthier properties



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### ABSTRACT

Dry-cured rabbit legs were produced with a 50% reduction or replacement of NaCl by KCl and with the addition of monosodium glutamate (MG). The effect of this reformulation on technological, nutritional, and sensory characteristics was evaluated. The sodium reformulation did not show a great impact on Aw, pH, weight loss, and volatile profile of the samples. The samples presented high protein (31.5 to 36.1%) and low fat contents (3.2 to 5.7%). In addition, all essential amino acids presented an amino acid score greater than 1.0. The reformulated samples showed a sodium reduction of 46.2% while the addition of KCl to the formulations provided a healthy Na/K ratio. Oleic acid was the major fatty acid (FA) (31.3% of total FA) and healthy lipid indexes were observed for all samples. Finally, the addition of MG was effective to compensate for the sensory defects caused by sodium reformulation.

### 1. Introduction

Rabbit meat has an excellent nutritional quality, due to its high oleic acid, proteins, and essential amino acids and low fat and cholesterol levels (Li et al., 2018). It also presents little allergenicity and contains significant amounts of minerals such as selenium and phosphorus, as well as B vitamins (Cullere et al., 2018; Cullere et al., 2019). It is worth mentioning that this meat can be even healthier by the addition of functional compounds to the animal diet, such as CLA, vitamin E, n-3 fatty acids, and selenium (Zotte & Szendrő, 2011). A recent study has shown that European consumers have a perception that wild game meats are beneficial for health, considering it more organic than other types of meat (Tomasevic et al., 2018).

However, several factors hinder the development of the rabbit meat production chain, including the absence of specialized industries, small investment in the area, and little research (Li et al., 2018). In addition, the public criticism regarding the welfare of rabbits reared for farming purposes (Matic et al., 2014) and the lower consumption of rabbit meat

has posed a serious threat to this market (Petracci, Soglia, & Leroy, 2018).

Therefore, new strategies are urgently needed to encourage the consumption of rabbit meat and guarantee the survival of this industry. Currently, there is a small variety of rabbit meat-based products. Thus, the use of this raw material for the development of innovative healthier meat products can be an effective strategy to attract new consumers, especially those concerned with health. In addition, processing can mask the typical wild flavor of rabbit meat that can result in purchase rejection by new consumers (Cullere & Zotte, 2018).

Within the wide range of processed meat products, dry-cured ham is considered a noble product from the meat industry that is traditionally made with pork leg, which gives a significant added value to the raw material (Bermúdez, Franco, Carballo, Sentandreu, & Lorenzo, 2014). This product is made by combining traditional knowledge and technology and is highly appreciated worldwide for its sensory and nutritional quality (Bermúdez, Franco, Carballo, & Lorenzo, 2014). One of the great advantages of dry-cured ham is its low bacterial counts due to

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limiting factors, such as its high salt content; the use of nitrate and nitrite; and the low water activity (Pérez-Santaescolástica et al., 2018; Toldrá, 2010). Furthermore, studies have shown that bioactive peptides with health benefits are generated during the ripening stage (Toldrá, Gallego, Reig, Aristoy, & Mora, 2020). Another great advantage is the appeal of clean label product, once it is usually contains only NaCl, nitrite, and nitrate in the formulations (Escudero et al., 2013; Gallego, Mora, & Toldrá, 2018).

However, dry-cured ham contains more than 5% NaCl (Armenteros, Aristoy, Barat, & Toldrá, 2012), thus the frequent and excessive consumption can contribute to an increase in the risk factors related to the prevalence of cardiovascular diseases in salt-sensitive individuals (Rico-Campà et al., 2020). Several strategies have been studied to reduce the sodium content of dry-cured ham (Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017), including the replacement of NaCl by KCl, which presents effective results (Armenteros, Toldrá, Aristoy, Ventanas, & Estévez, 2012; Petit et al., 2019). The main limitation of the KCl addition is the increase in processing time due to the greater difficulty of KCl to penetrate into the product (Aliño, Grau, Toldrá, & Barat, 2010; Blesa et al., 2008). In addition, it is well documented that KCl can impart a bitter, metallic, and an astringent taste to the product when used at replacement levels higher than 50% of NaCl (Da Silva et al., 2020; Santos Alves et al., 2017). A previous study of our research group demonstrated that the use of flavor enhancers, such as monosodium glutamate, can be effective to reduce the sensory defects caused by the addition of high KCl concentrations to fermented cooked sausages (Dos Santos, Campagnol, Morgano, & Pollonio, 2014).

However, so far, the use of rabbit meat for the development of dry-cured products with healthier appeal has been little explored. In this framework, this study aimed to evaluate the effect of the reduction or replacement of 50% NaCl by KCl as well as the addition of monosodium glutamate on the technological, nutritional, and sensory characteristics of dry-cured rabbit leg.

## 2. Materials and methods

### 2.1. Selection of raw rabbit leg

One hundred and thirty raw legs with an average weight of  $212.1 \pm 27.5$  g from New Zealand White rabbits (*Oryctolagus cuniculus*) reared in similar systems were provided by a local slaughterhouse. Rabbits (4-month-old and average weight of  $3238.3 \pm 419.5$  g) were electrically stunned and slaughtered according to the standards described by the Brazilian legislation (MAPA, 2000). The carcasses were stored in a cold chamber with an average temperature of  $2 \pm 1$  °C for entering rigor mortis. Only the legs with pH values between 5.40 and 5.70 within 2 h post mortem interval were selected (Toldrá, 2010). After 12 h of storage, the legs were removed from the carcasses and massaged by hand in running water at room temperature, for approximately 1 min, to remove any blood residue present on the piece. Then, the legs were hung for dripping for 2 min. All legs were vacuum-packed and stored at  $-18$  °C for 30 days. The raw legs presented the following proximate composition ( $n = 5$ ):  $68.5 \pm 2.9\%$  moisture;  $22.2 \pm 0.5\%$  protein;  $2.2 \pm 0.4\%$  fat; and  $1.2 \pm 0.08\%$  ash (AOAC, 2006).

### 2.2. Manufacture of dry-cured rabbit leg

The legs were thawed in a cold chamber for 3 days at  $2 \pm 1$  °C and 80–90% RH. The raw legs were randomly assigned to the five treatment groups. The control (NaCl<sub>100%</sub>) was made with 4% NaCl, which is the amount commonly used in similar products such as dry-cured ham (Ripollés, Campagnol, Armenteros, Aristoy, & Toldrá, 2011). In the modified treatments, the NaCl content was reduced by 50% and replaced by KCl and monosodium glutamate (Table 1). For all treatments, 100 ppm of NaNO<sub>2</sub> and 200 ppm of NaNO<sub>3</sub> were used. The amount of the ingredients was determined according to the weight of

**Table 1**  
Formulations of low-sodium dry cured rabbit leg.

| (%)             | Na <sub>100%</sub> | Na <sub>50%</sub> | Na <sub>50%</sub> K <sub>50%</sub> | Na <sub>50%</sub> G | Na <sub>50%</sub> K <sub>50%</sub> G |
|-----------------|--------------------|-------------------|------------------------------------|---------------------|--------------------------------------|
| NaCl            | 4.0                | 2.0               | 2.0                                | 2.0                 | 2.0                                  |
| KCl             | –                  | –                 | 2.0                                | –                   | 2.0                                  |
| MG <sup>a</sup> | –                  | –                 | –                                  | 0.3                 | 0.3                                  |

<sup>a</sup> monosodium glutamate.

each leg. The ingredients were mixed manually in each leg until uniform distribution. After salting, the legs were hung and kept in a cold chamber at  $2 \pm 1$  °C and 85–95% RH for 7 days until uniform salt distribution. After this period, the legs were maintained at  $12 \pm 1$  °C and 65–75% RH until the 28th day of manufacture.

### 2.3. Sampling

*Biceps femoris* (BF) muscles were removed from legs at different times from the beginning of the process: 1, 7, 14, 21 and 28 days. pH and Aw were determined during the manufacture period of the legs (days 1, 7, 14, 21, and 28). The other instrumental and sensory analyzes were performed at the end of the manufacturing process (day 28). The determination of pH, Aw, and the sensory evaluation was performed right after the BF removal. For the other instrumental analyses, the BF samples were sampled immediately after the end of manufacturing process (28 days), vacuum packed, and stored at  $-26$  °C for further analysis. Instrumental measurements were performed in triplicate using three legs per treatment for each day of sampling. The sensory evaluation was performed using 8 legs per treatment.

### 2.4. Aw and pH

Aw was measured using an Aqualab 4TE apparatus (Decagon, Pullman, USA) previously calibrated according to the manufacturer's instructions. The pH was determined in the sample diluted 1:10 (BF: water) using a digital pH meter with a glass electrode (Digimed - DM-23 DC, São Paulo, SP, Brazil).

### 2.5. Weight loss

Ten legs of each treatment were sampled and weighed immediately after salting (day 1) and after 7, 14, 21, and 28 days of the manufacturing process. The weight loss was calculated by the difference between the initial weight and the weight determined weekly.

### 2.6. Physicochemical characterization and Na and K levels

The moisture (AOAC Method 950.46), fat (AOAC Method 991.36), protein (AOAC Method 2011.04), and ash (AOAC Method 920.153) contents of the BF were determined according to the procedures described by AOAC (2006). The sodium and potassium levels were determined in the BF by inductively coupled plasma - optical emission spectroscopy (ICP-OES) using a Thermo-Fisher ICAP 6000 plasma emission spectrometer (Thermo-Fisher, Cambridge, UK) as described by Lorenzo et al. (2015).

### 2.7. Amino acid profile

The amino acid profile was determined as described by Domínguez, Crecente, Borrajo, Agregán, and Lorenzo (2015). For the protein hydrolysis, 0.1 g of sample and 5 mL of hydrochloric acid (6 N) were placed in an ampoule glass. The ampoules were closed and maintained at 110 °C for 24 h. The determination of tryptophan was not possible, once it is converted into ammonium during the acid hydrolysis (Lorenzo et al., 2011). After protein hydrolysis, the sample was mixed with 200 mL of distilled water and filtered through a 45 µm filter. Standard and

samples were subjected to derivatization using the commercial kit AccQ-Fluor reagent. For that, Acc-Fluor borate buffer pH 8.8 was added to 10 µL sample to make 100 µL of solution. Afterward, 20 µL of AccQ-Fluor reagent was mixed until the rapid derivatization of all primary and secondary amines. A high-performance liquid chromatograph (Alliance 2695 model, Waters, Milford, MA) equipped with a scanning fluorescence detector (model 2475, Waters) was used to separate the derivatized amino acids, using a Waters AccQ-Tag column (3.9 × 150 mm, with a particle size of 3 µm). The flow rate was 1.0 mL/min at 37 °C, and the excitation and emission wavelengths were 250 and 395 nm, respectively. The AccQ-Tag amino acid analysis protocol (Waters Corporation) was used to determine the mobile phase and the gradient composition. The identification of the amino acids was performed by comparing the retention times, and the quantification was performed by the external standard method using the Standard Amino Acid H (Thermo Scientific, Rockford, IL, USA). The results were expressed in g/100 g of protein. Amino acid score (AAS) was determined for each essential amino acid using Eq. (1) considering the recommended amino acid scoring pattern for adults (FAO/WHO/UNU, 2007).

$$AAS = \frac{\text{Content of essential amino acids in the sample}}{\text{Content of essential amino acids in the reference protein}} \quad (1)$$

## 2.8. Fatty acids profile

The fat was extracted from BF using the method of Bligh and Dyer (1959) and the fatty acid methyl esters (FAME) were obtained according to the procedures described by Hartman and Lago (1973). The separation and quantification of FAME were performed using a gas chromatograph (GC-Agilent 7890B; Agilent Technologies Spain, SL, Madrid, Spain) equipped with a flame ionization detector and an HP 7683 automatic sample injector. A Supelco SPTM-2560 fused silica capillary column (100 m, 0.25 mm i.d., 0.2 µm film thickness; Supelco Inc., Bellefonte, PA, USA) was used. The chromatographic conditions were performed following the procedure described by Domínguez et al. (2015). For the FAME identification, the retention times of the sample constituents were compared with FAME standards, and the results were expressed in g/100 g of the total fatty acids.

## 2.9. Volatile compounds

The solid-phase microextraction (SPME) methodology described by Domínguez et al. (2019) was used for the extraction of the volatile compounds. The headspace extraction, the conditioning, and the injection of the samples were performed using an automatic sampler, model PAL RTC 120 (CTC Analytics AG, Zwingen, Switzerland), and 1 g of minced BF in a vial of 20 mL. To ensure a constant temperature in the samples and the headspace, temperature equilibrium was carried out at 37 °C, similar to the temperature used in the extraction procedures, except for the time, which was 15 min. At the end of the extraction, the fiber was placed in the injection port (7890B gas chromatograph Agilent Technologies, Santa Clara, USA) equipped with an MS77 5977B mass detector. The separation of volatile compounds was performed using a DB-624 capillary column (30 m, 250 µm id, 1.4 µm film thickness; J&W Scientific, Folsom, CA, USA).

The identification of compounds was performed by comparing their mass spectra with those of the NIST14 library (National Institute of Standards and Technology, Gaithersburg, USA) and/or by comparing mass spectra and retention time with authentic standards (Supelco, Bellefonte, USA) and/or calculating the relative retention index using a C5-C14 n-alkanes series (Supelco 44,585-U, Supelco, Bellefonte, USA) and comparing with literature data. The results were expressed as area units (AU) × 10<sup>5</sup>/g of the sample.

## 2.10. Sensory analysis

The sensory technique called sorting task was used to characterize sensorially the dry-cured rabbit leg manufactured with different NaCl levels. This method is based on the categorization of products according to the global similarity. This process is more intuitive than the conventional descriptive methods since it is used routinely by individuals (Chollet, Lelièvre, Abdi, & Valentin, 2011; Gómez-Corona, Chollet, Escalona-Buendía, & Valentin, 2017). To do so, a total of 110 (64 women and 46 men) regular consumers of dry-cured meat products with age ranging from 18 to 64 years old, were conditioned in sensory booths to participated in the analysis. All BF samples were simultaneously served to the participants in slices (~5 g) at 8 °C, conditioned in plastic cups coded with 3-digit random number. First, the participants were asked to taste each sample in a balanced way and then to group them based on its similarity, but non-specific criterion was provided to perform the test. In addition, participants were free to make as many groups as they wanted. After completing the sorting task, the individuals were asked to write a few words to describe each of these groups. After each evaluation, they were asked to rinse their mouth with water and crackers. This study was approved by the Human Research Ethics Committee of the Federal University of Santa Maria (CAAE: 16218519.0.0000.5346) and all participants signed an informed consent form agreeing to participate in the research.

## 2.11. Data analysis

### 2.11.1. Instrumental data

The instrumental data were analyzed through analysis of variance (ANOVA), using three legs per batch, in triplicate. The different salt concentrations were considered as a fixed effect and leg was considered as a random effect. Tukey's test at the level of 5% ( $P < 0.05$ ) of significance was used to compare the means between treatments. Data were analyzed using the SPSS statistical program (SPSS, Chicago, IL, USA).

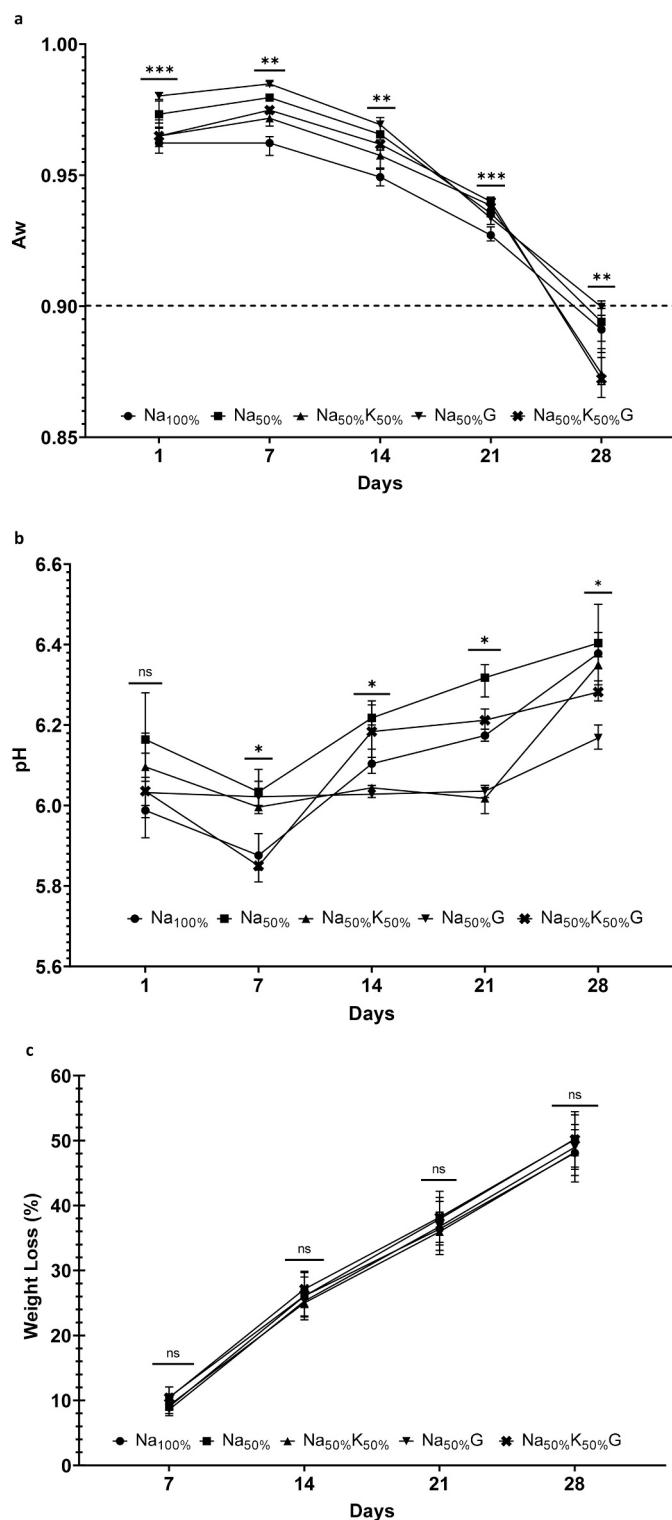
### 2.11.2. Sensory data

A global similarity matrix (obtained by aggregating the data with the participants in the columns and the BF samples in the rows) was submitted to the DISTATIS technique which is a generalization of multidimensional scaling to obtain a products space considering the individual variability of individuals expressed as confidence ellipses (Abdi, Valentin, Chollet, & Chrea, 2007). The free sensory descriptions of samples were lemmatized and categorized in a lexical table with semantic similarity (Rios-Mera et al., 2019). Subsequently, this lexical table was analyzed by correspondence analysis (CA) arranging the sensory descriptors in the columns and samples in the rows. These analyses were performed in R environment, using packages ExPosition and DistatisR (Beaton, Fatt, & Abdi, 2014) for DISTATIS and FactoMineR for CA.

## 3. Results and discussion

### 3.1. Aw, pH, and weight loss

The manufacturing process was completed on the 28th day of storage when all samples reached Aw values below 0.90 (Brasil, 2000) (Fig. 1a). As expected, the samples with the higher salt concentrations (Na<sub>100%</sub>, Na<sub>50%</sub>K<sub>50%</sub>, and Na<sub>50%</sub>K<sub>50%</sub>G) showed a greater decrease in Aw values. A similar trend was reported by Petričević, Radović, Lukić, Listić, and Medić (2018), who evaluated dry-cured ham with different salt concentrations. Studies have shown that potassium has greater difficulty to penetrate into the product when compared with sodium, which increases the manufacture time of dry-cured ham (Aliño et al., 2010; Blesa et al., 2008). In this study, this result was not observed probably due to the small thickness of the rabbit leg. In addition, the samples made with the addition of KCl had a lower Aw when compared with the sample Na<sub>100%</sub> (0.89; 0.87; and 0.87 for Na<sub>100%</sub>, Na<sub>50%</sub>K<sub>50%</sub>, and Na<sub>50%</sub>K<sub>50%</sub>G,



**Fig. 1.** Aw (1a), pH (1b), and weight loss (1c) of low-sodium dry-cured rabbit leg. Batches: Described in Table 1. \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ), \*\*\* ( $P < 0.001$ ), n.s. (not significant).

respectively,  $P < 0.01$ , which is a positive result, as it guarantees greater safety and microbiological stability of the product (Aliño et al., 2010; Inguglia et al., 2017). In agreement with the results found in this study, Vidal et al. (2019) reported that replacing 50% NaCl with KCl did not change the moisture content of jerked beef, however, Aw was reduced in products with KCl.

The pH values of the samples at the beginning of the manufacture

(day 1) ranged from 5.9 to 6.1 ( $P > 0.05$ ) (Fig. 1b). A reduction of pH values was observed during the first seven days of manufacture probably due to the acid production by lactic acid bacteria from the contaminating flora of rabbit leg (Lorenzo, Fontán, Cachaldora, Franco, & Carballo, 2010; Martínez-Onandi, Sánchez, Nuñez, & Picon, 2019). From the 7th day of manufacture, there was an increase in pH values due to the accumulation of alkaline compounds from the proteolytic reactions (Pinna, Saccani, Schivazappa, Simoncini, & Virgili, 2020). The sample Na<sub>50%</sub> presented the greatest increase in pH values ( $P < 0.05$ ). This result can be attributed to the activation of muscle proteases caused by salt reduction, which increased the proteolytic activity and the content of basic free amino acids (dos Santos et al., 2015). At the end of the manufacture, the pH values ranged from 6.1 to 6.3 ( $P < 0.05$ ), which is expected for this class of meat products (Bou, Llauger, Arnau, Olmos, & Fulladosa, 2020; Zhang et al., 2020).

A progressive increase in weight loss was observed in the samples during the manufacture due to the drying process (Fig. 1c), with a final weight loss close to 50% ( $P > 0.05$ ). This value is higher than that found by other authors in dry-cured hams (Bou et al., 2020; Čandek-Potokar et al., 2020; Coll-Brasas et al., 2019; López-Pedrouso et al., 2019), which may be due to the low fat content (2.2%) and higher moisture content (68.5%) of rabbit meat leg. The visual aspect of the samples throughout the manufacturing period is shown in Fig. S1.

### 3.2. Proximate composition and mineral levels

The results of the proximate composition and the mineral contents of the low-sodium dry-cured rabbit leg are shown in Table 2. The moisture content of the samples ranged from 42.3 to 44.5%, which are similar to those found in other types of dry-cured meat products (Cittadini et al., 2019; Lorenzo et al., 2011; Lorenzo & Carballo, 2016; Petričević et al., 2018). As also reported by Armenteros, Aristoy, et al. (2012) and Wu et al. (2015) in similar products, the replacement of 50% NaCl by KCl did not affect ( $P > 0.05$ ) the moisture content of the samples. The sample Na<sub>100%</sub> showed a lower moisture level ( $P < 0.01$ ) when compared with the samples with a 50% salt reduction and without the addition of KCl (Na<sub>50%</sub> and Na<sub>50%</sub>G). These results can be explained by the diffusivity theory since the higher the salt diffusion into the product, the greater the moisture loss during drying (Martuscelli, Lupieri, Sacchetti, Mastrolola, & Pittia, 2017).

As expected, the samples with higher moisture content presented lower protein and fat levels ( $P < 0.05$ ). It is worth emphasizing that the samples presented fat levels (3.2 to 5.7%) close to the maximum limit (3 g fat per 100 g) established by the European legislation to be claimed as a low-fat product. In addition, all samples exhibited high protein levels (31.5 to 36.1%), thus they can be claimed as high in protein, once more than 20% of the energy value is provided by protein (European Parliament, 2006).

The differences in the ash contents of the samples were consistent with the salt concentrations of each treatment (Table 2). The sample Na<sub>100%</sub> presented 2.3% sodium content, which is similar to that reported by other authors for dry-cured pork hams (Betoli et al., 2020; Campos, Mussons, Antolin, Debán, & Pardo, 2017). The 50% salt reduction reduced the sodium content by 46.2%, thus the samples can be claimed as “reduced in sodium” (European Parliament, 2006). The use of KCl also led to an increase in potassium content by 200%. These results had a major impact on the sodium-to-potassium ratio (Na/K) of the samples, which is considered a more reliable indicator to assess the risks of cardiovascular disease (CVD) and CVD-related mortality when compared with the intake of sodium or potassium alone (Bailey, Tahraní, & Barnett, 2016). The World Health Organization recommends a diet with a Na/K ratio  $< 1.0$  to reduce the risk factors related to the onset of cardiovascular diseases (WHO, 2003). In this study, the sample Na<sub>100%</sub> showed the highest ( $P < 0.001$ ) Na/K ratio (3.6). Although the samples with NaCl reduction and without the addition of KCl (Na<sub>50%</sub> and Na<sub>50%</sub>G) showed a decrease of 47% in Na/K ratio when compared to Na<sub>100%</sub>,

**Table 2**

Proximate composition, sodium and potassium levels, and Na/K ratio of low-sodium dry-cured rabbit leg.

|                      | Na <sub>100%</sub>   | Na <sub>50%</sub>    | Na <sub>50%</sub> K <sub>50%</sub> | Na <sub>50%</sub> G  | Na <sub>50%</sub> K <sub>50%</sub> G | SEM  | SIG |
|----------------------|----------------------|----------------------|------------------------------------|----------------------|--------------------------------------|------|-----|
| Moisture (%)         | 42.34 <sup>b</sup>   | 44.19 <sup>a</sup>   | 42.89 <sup>ab</sup>                | 44.53 <sup>a</sup>   | 42.42 <sup>b</sup>                   | 0.28 | **  |
| Protein (%)          | 36.15 <sup>a</sup>   | 32.79 <sup>b</sup>   | 31.52 <sup>b</sup>                 | 34.31 <sup>ab</sup>  | 35.90 <sup>a</sup>                   | 0.55 | *   |
| Fat (%)              | 5.10 <sup>a</sup>    | 3.28 <sup>b</sup>    | 5.78 <sup>a</sup>                  | 3.33 <sup>b</sup>    | 4.30 <sup>ab</sup>                   | 0.28 | *   |
| Ash (%)              | 14.93 <sup>a</sup>   | 11.60 <sup>b</sup>   | 15.06 <sup>a</sup>                 | 12.02 <sup>b</sup>   | 15.01 <sup>a</sup>                   | 0.52 | *** |
| Sodium (mg/100 g)    | 2288.05 <sup>a</sup> | 1247.85 <sup>b</sup> | 1232.98 <sup>b</sup>               | 1245.75 <sup>b</sup> | 1204.63 <sup>b</sup>                 | 50.2 | *** |
| Potassium (mg/100 g) | 633.84 <sup>b</sup>  | 635.33 <sup>b</sup>  | 1946.67 <sup>a</sup>               | 639.47 <sup>b</sup>  | 1945.76 <sup>a</sup>                 | 71.5 | *** |
| Na/K ratio           | 3.63 <sup>a</sup>    | 1.95 <sup>b</sup>    | 0.65 <sup>c</sup>                  | 1.92 <sup>b</sup>    | 0.62 <sup>c</sup>                    | 0.01 | *** |

a-c Mean values in the same row (corresponding to the same parameter) not followed by a common letter differ significantly ( $P < 0.05$ ).

Batches: Described in Table 1.

SEM: standard error of the mean; Sig.: significance: \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ), \*\*\* ( $P < 0.001$ ), n.s. (not significant).

they presented values above the recommended limit. In contrast, the replacement of 50% NaCl with KCl in the sample Na<sub>100%</sub> was effective in reducing the Na/K ratio to healthy levels (3.6; 0.65; and 0.62 for Na<sub>100%</sub>, Na<sub>50%</sub> K<sub>50%</sub>, and Na<sub>50%</sub> K<sub>50%</sub> G, respectively,  $P < 0.001$ ).

### 3.3. Amino acids profile

The amino acid composition of the dry-cured rabbit leg was not affected ( $P > 0.05$ ) by the reduction or replacement of 50% of NaCl by KCl and the addition of MG (Table 3). The total non-essential amino acids (NEAA) and essential amino acids (EAA) levels of the samples were close to 53 and 47 g/100 g protein, respectively. In quantitative terms, the main NEAA detected were glutamic acid and aspartic acid, while the main EAA were lysine and leucine. A similar result was reported by Nasr, Abd-Elhamid, and Hussein (2017), who studied the amino-acid profile of different rabbit breeds and their crosses. It is worth noting that the protein of the samples can be considered to be of high nutritional quality, once they contained more than 30% of EAA (Paddon-Jones & Rasmussen, 2009). In addition, the amino acid score of all EAA was greater than 1.0 (Table 4), thus the EAA levels were higher than the amino acid scoring pattern recommended for adults by FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition (WHO, 2007).

**Table 3**

Amino acid composition (g/100 g of protein) of the low-sodium dry-cured rabbit leg.

| Amino acids              | Na <sub>100%</sub> | Na <sub>50%</sub>  | Na <sub>50%</sub> K <sub>50%</sub> | Na <sub>50%</sub> G | Na <sub>50%</sub> K <sub>50%</sub> G | SEM  | SIG  |
|--------------------------|--------------------|--------------------|------------------------------------|---------------------|--------------------------------------|------|------|
| <b>Non-essential</b>     |                    |                    |                                    |                     |                                      |      |      |
| Hydroxyproline           | 0.43 <sup>a</sup>  | 0.18 <sup>a</sup>  | 0.04 <sup>a</sup>                  | 0.10 <sup>a</sup>   | 0.27 <sup>a</sup>                    | 0.01 | n.s. |
| Aspartic acid            | 9.51 <sup>a</sup>  | 9.74 <sup>a</sup>  | 9.62 <sup>a</sup>                  | 9.61 <sup>a</sup>   | 9.30 <sup>a</sup>                    | 0.21 | n.s. |
| Serine                   | 4.14 <sup>a</sup>  | 4.17 <sup>a</sup>  | 4.32 <sup>a</sup>                  | 4.11 <sup>a</sup>   | 4.47 <sup>a</sup>                    | 0.21 | n.s. |
| Glutamic acid            | 15.81 <sup>a</sup> | 16.23 <sup>a</sup> | 16.21 <sup>a</sup>                 | 16.71 <sup>a</sup>  | 16.23 <sup>a</sup>                   | 0.33 | n.s. |
| Glycine                  | 6.03 <sup>a</sup>  | 5.31 <sup>a</sup>  | 5.20 <sup>a</sup>                  | 5.37 <sup>a</sup>   | 5.64 <sup>a</sup>                    | 0.53 | n.s. |
| Arginine                 | 6.87 <sup>a</sup>  | 6.97 <sup>a</sup>  | 7.02 <sup>a</sup>                  | 6.89 <sup>a</sup>   | 7.09 <sup>a</sup>                    | 0.10 | n.s. |
| Alanine                  | 6.18 <sup>a</sup>  | 6.19 <sup>a</sup>  | 6.07 <sup>a</sup>                  | 6.32 <sup>a</sup>   | 6.18 <sup>a</sup>                    | 0.12 | n.s. |
| Proline                  | 4.10 <sup>a</sup>  | 3.75 <sup>a</sup>  | 3.78 <sup>a</sup>                  | 3.78 <sup>a</sup>   | 3.83 <sup>a</sup>                    | 0.23 | n.s. |
| $\Sigma$ NEAA            | 53.1 <sup>a</sup>  | 52.5 <sup>a</sup>  | 52.3 <sup>a</sup>                  | 52.9 <sup>a</sup>   | 53.0 <sup>a</sup>                    | 0.7  | n.s. |
| <b>Essential</b>         |                    |                    |                                    |                     |                                      |      |      |
| Histidine                | 2.85 <sup>a</sup>  | 2.99 <sup>a</sup>  | 3.06 <sup>a</sup>                  | 2.98 <sup>a</sup>   | 3.11 <sup>a</sup>                    | 0.15 | n.s. |
| Isoleucine               | 5.07 <sup>a</sup>  | 5.05 <sup>a</sup>  | 5.13 <sup>a</sup>                  | 5.05 <sup>a</sup>   | 4.94 <sup>a</sup>                    | 0.10 | n.s. |
| Leucine                  | 8.30 <sup>a</sup>  | 8.36 <sup>a</sup>  | 8.44 <sup>a</sup>                  | 8.35 <sup>a</sup>   | 8.15 <sup>a</sup>                    | 0.16 | n.s. |
| Lysine                   | 8.72 <sup>a</sup>  | 8.98 <sup>a</sup>  | 8.93 <sup>a</sup>                  | 9.00 <sup>a</sup>   | 8.53 <sup>a</sup>                    | 0.29 | n.s. |
| Methionine + cysteine    | 3.63 <sup>a</sup>  | 3.57 <sup>a</sup>  | 3.50 <sup>a</sup>                  | 3.43 <sup>a</sup>   | 3.59 <sup>a</sup>                    | 0.11 | n.s. |
| Methionine               | 2.87 <sup>a</sup>  | 2.74 <sup>a</sup>  | 2.72 <sup>a</sup>                  | 2.71 <sup>a</sup>   | 2.85 <sup>a</sup>                    | 0.10 | n.s. |
| Cysteine                 | 0.76 <sup>a</sup>  | 0.83 <sup>a</sup>  | 0.78 <sup>a</sup>                  | 0.72 <sup>a</sup>   | 0.73 <sup>a</sup>                    | 0.05 | n.s. |
| Phenylalanine + tyrosine | 8.28 <sup>a</sup>  | 8.37 <sup>a</sup>  | 8.39 <sup>a</sup>                  | 8.14 <sup>a</sup>   | 8.24 <sup>a</sup>                    | 0.17 | n.s. |
| Phenylalanine            | 4.47 <sup>a</sup>  | 4.46 <sup>a</sup>  | 4.51 <sup>a</sup>                  | 4.44 <sup>a</sup>   | 4.45 <sup>a</sup>                    | 0.09 | n.s. |
| Tyrosine                 | 3.81 <sup>a</sup>  | 3.90 <sup>a</sup>  | 3.88 <sup>a</sup>                  | 3.71 <sup>a</sup>   | 3.79 <sup>a</sup>                    | 0.10 | n.s. |
| Threonine                | 4.63 <sup>a</sup>  | 4.70 <sup>a</sup>  | 4.76 <sup>a</sup>                  | 4.65 <sup>a</sup>   | 4.84 <sup>a</sup>                    | 0.11 | n.s. |
| Valine                   | 5.46 <sup>a</sup>  | 5.43 <sup>a</sup>  | 5.54 <sup>a</sup>                  | 5.51 <sup>a</sup>   | 5.60 <sup>a</sup>                    | 0.09 | n.s. |
| $\Sigma$ EAA             | 46.9 <sup>a</sup>  | 47.5 <sup>a</sup>  | 47.7 <sup>a</sup>                  | 47.1 <sup>a</sup>   | 47.0 <sup>a</sup>                    | 0.7  | n.s. |

Batches: Described in Table 1.

NEAA: Non-essential amino acids; EAA: Essential amino acids.

<sup>a</sup> Mean values in the same row followed by a common letter not differ significantly ( $P > 0.05$ ). SEM: standard error of the mean; Sig.: significance: n.s. (not significant).

**Table 4**

Amino acid score of the low-sodium dry-cured rabbit leg.

|                          | Na <sub>100%</sub> | Na <sub>50%</sub> | Na <sub>50%</sub> K <sub>50%</sub> | Na <sub>50%</sub> G | Na <sub>50%</sub> K <sub>50%</sub> G | SEM               | SIG  |
|--------------------------|--------------------|-------------------|------------------------------------|---------------------|--------------------------------------|-------------------|------|
| Histidine                | 1.9 <sup>a</sup>   | 2.0 <sup>a</sup>  | 2.0 <sup>a</sup>                   | 2.0 <sup>a</sup>    | 2.1 <sup>a</sup>                     | 0.10 <sup>b</sup> | n.s. |
| Isoleucine               | 1.7 <sup>a</sup>   | 1.7 <sup>a</sup>  | 1.7 <sup>a</sup>                   | 1.7 <sup>a</sup>    | 1.6 <sup>a</sup>                     | 0.03              | n.s. |
| Leucine                  | 1.4 <sup>a</sup>   | 1.4 <sup>a</sup>  | 1.4 <sup>a</sup>                   | 1.4 <sup>a</sup>    | 1.4 <sup>a</sup>                     | 0.03              | n.s. |
| Lysine                   | 1.9 <sup>a</sup>   | 2.0 <sup>a</sup>  | 2.0 <sup>a</sup>                   | 2.0 <sup>a</sup>    | 1.9 <sup>a</sup>                     | 0.07              | n.s. |
| Methionine + Cysteine    | 1.7 <sup>a</sup>   | 1.6 <sup>a</sup>  | 1.6 <sup>a</sup>                   | 1.6 <sup>a</sup>    | 1.6 <sup>a</sup>                     | 0.05              | n.s. |
| Methionine               | 1.8 <sup>a</sup>   | 1.7 <sup>a</sup>  | 1.7 <sup>a</sup>                   | 1.7 <sup>a</sup>    | 1.8 <sup>a</sup>                     | 0.07              | n.s. |
| Cysteine                 | 1.3 <sup>a</sup>   | 1.4 <sup>a</sup>  | 1.3 <sup>a</sup>                   | 1.2 <sup>a</sup>    | 1.2 <sup>a</sup>                     | 0.09              | n.s. |
| Phenylalanine + tyrosine | 2.2 <sup>a</sup>   | 2.2 <sup>a</sup>  | 2.2 <sup>a</sup>                   | 2.1 <sup>a</sup>    | 2.2 <sup>a</sup>                     | 0.04              | n.s. |
| Threonine                | 2.0 <sup>a</sup>   | 2.0 <sup>a</sup>  | 2.1 <sup>a</sup>                   | 2.0 <sup>a</sup>    | 2.1 <sup>a</sup>                     | 0.05              | n.s. |
| Valine                   | 1.4 <sup>a</sup>   | 1.4 <sup>a</sup>  | 1.4 <sup>a</sup>                   | 1.4 <sup>a</sup>    | 1.4 <sup>a</sup>                     | 0.02              | n.s. |

Batches: Described in Table 1.

SEM: standard error of the mean; Sig.: significance: n.s. (not significant).

<sup>a</sup> Mean values in the same row followed by a common letter not differ significantly ( $P > 0.05$ ).<sup>b</sup> Values of amino acid score were calculated using the recommended amino acid scoring pattern for adult (FAO/WHO/UNU, 2007). The indispensable amino acids reference patterns are expressed as g amino acid/g protein: Histidine: 1.5; Isoleucine: 3; Leucine: 5.9; Lysine: 4.5; Methionine + cysteine: 2.2; Methionine: 1.6; Cysteine: 0.6; Phenylalanine + tyrosine: 3.8; Threonine: 2.3; Valine: 3.9.**Table 5**

Fatty acids profile (expressed as g/100 g of fatty acids) of low-sodium dry-cured rabbit leg.

|                | Na <sub>100%</sub> | Na <sub>50%</sub>  | Na <sub>50%</sub> K <sub>50%</sub> | Na <sub>50%</sub> G | Na <sub>50%</sub> K <sub>50%</sub> G | SEM  | SIG  |
|----------------|--------------------|--------------------|------------------------------------|---------------------|--------------------------------------|------|------|
| C10:0          | 0.07 <sup>a</sup>  | 0.08 <sup>a</sup>  | 0.08 <sup>a</sup>                  | 0.07 <sup>a</sup>   | 0.06 <sup>a</sup>                    | 0.01 | n.s. |
| C12:0          | 0.08 <sup>a</sup>  | 0.09 <sup>a</sup>  | 0.08 <sup>a</sup>                  | 0.08 <sup>a</sup>   | 0.07 <sup>a</sup>                    | 0.01 | n.s. |
| C14:0          | 2.15 <sup>a</sup>  | 2.19 <sup>a</sup>  | 2.45 <sup>a</sup>                  | 2.16 <sup>a</sup>   | 2.30 <sup>a</sup>                    | 0.14 | n.s. |
| C14:1n-5       | 0.72 <sup>a</sup>  | 0.48 <sup>a</sup>  | 0.50 <sup>a</sup>                  | 0.58 <sup>a</sup>   | 0.52 <sup>a</sup>                    | 0.05 | n.s. |
| C15:0          | 0.39 <sup>a</sup>  | 0.42 <sup>a</sup>  | 0.45 <sup>a</sup>                  | 0.44 <sup>a</sup>   | 0.42 <sup>a</sup>                    | 0.02 | n.s. |
| C15:1n-5       | 0.70 <sup>a</sup>  | 0.72 <sup>a</sup>  | 0.58 <sup>a</sup>                  | 0.62 <sup>a</sup>   | 0.67 <sup>a</sup>                    | 0.03 | n.s. |
| C16:0          | 23.59 <sup>a</sup> | 23.89 <sup>a</sup> | 24.85 <sup>a</sup>                 | 23.56 <sup>a</sup>  | 24.75 <sup>a</sup>                   | 0.49 | n.s. |
| C16:1n-7       | 8.04 <sup>a</sup>  | 6.17 <sup>a</sup>  | 6.18 <sup>a</sup>                  | 6.74 <sup>a</sup>   | 6.37 <sup>a</sup>                    | 0.28 | n.s. |
| C17:0          | 0.47 <sup>b</sup>  | 0.50 <sup>b</sup>  | 0.53 <sup>a</sup>                  | 0.54 <sup>a</sup>   | 0.50 <sup>b</sup>                    | 0.01 | *    |
| C17:1n-7       | 0.38 <sup>a</sup>  | 0.38 <sup>a</sup>  | 0.41 <sup>a</sup>                  | 0.39 <sup>a</sup>   | 0.37 <sup>a</sup>                    | 0.01 | n.s. |
| C18:0          | 6.26 <sup>a</sup>  | 6.62 <sup>a</sup>  | 6.53 <sup>a</sup>                  | 6.81 <sup>a</sup>   | 6.49 <sup>a</sup>                    | 0.09 | n.s. |
| 9 t-C18:1      | 0.13 <sup>a</sup>  | 0.13 <sup>a</sup>  | 0.14 <sup>a</sup>                  | 0.13 <sup>a</sup>   | 0.15 <sup>a</sup>                    | 0.01 | n.s. |
| 11 t-C18:1     | 0.08 <sup>a</sup>  | 0.09 <sup>a</sup>  | 0.08 <sup>a</sup>                  | 0.08 <sup>a</sup>   | 0.11 <sup>a</sup>                    | 0.01 | n.s. |
| C18:1n-9       | 27.83 <sup>a</sup> | 27.49 <sup>a</sup> | 28.66 <sup>a</sup>                 | 28.07 <sup>a</sup>  | 27.55 <sup>a</sup>                   | 0.29 | n.s. |
| C18:1n-7       | 1.98 <sup>ab</sup> | 1.70 <sup>b</sup>  | 1.69 <sup>b</sup>                  | 1.93 <sup>ab</sup>  | 2.16 <sup>ab</sup>                   | 0.06 | *    |
| 9 t,11 t-C18:2 | 0.19 <sup>b</sup>  | 0.14 <sup>c</sup>  | 0.16 <sup>bc</sup>                 | 0.18 <sup>b</sup>   | 0.23 <sup>a</sup>                    | 0.01 | *    |
| C18:2n-6       | 21.98 <sup>a</sup> | 23.25 <sup>a</sup> | 21.92 <sup>a</sup>                 | 22.54 <sup>a</sup>  | 21.99 <sup>a</sup>                   | 0.35 | n.s. |
| C18:3n-6       | 0.06 <sup>a</sup>  | 0.08 <sup>a</sup>  | 0.07 <sup>a</sup>                  | 0.07 <sup>a</sup>   | 0.06 <sup>a</sup>                    | 0    | n.s. |
| C18:3n-3       | 1.69 <sup>a</sup>  | 1.59 <sup>a</sup>  | 1.64 <sup>a</sup>                  | 1.65 <sup>a</sup>   | 1.58 <sup>a</sup>                    | 0.03 | n.s. |
| 9c,11 t-C18:2  | 0.06 <sup>a</sup>  | 0.06 <sup>a</sup>  | 0.05 <sup>a</sup>                  | 0.06 <sup>a</sup>   | 0.06 <sup>a</sup>                    | 0    | n.s. |
| C20:0          | 0.10 <sup>a</sup>  | 0.10 <sup>a</sup>  | 0.10 <sup>a</sup>                  | 0.10 <sup>a</sup>   | 0.11 <sup>a</sup>                    | 0    | n.s. |
| C20:1n-9       | 0.38 <sup>a</sup>  | 0.36 <sup>a</sup>  | 0.36 <sup>a</sup>                  | 0.39 <sup>a</sup>   | 0.46 <sup>a</sup>                    | 0.02 | n.s. |
| C20:2n-6       | 0.26 <sup>a</sup>  | 0.29 <sup>a</sup>  | 0.24 <sup>a</sup>                  | 0.29 <sup>a</sup>   | 0.31 <sup>a</sup>                    | 0.01 | n.s. |
| C20:3n-6       | 0.22 <sup>a</sup>  | 0.27 <sup>a</sup>  | 0.19 <sup>a</sup>                  | 0.22 <sup>a</sup>   | 0.25 <sup>a</sup>                    | 0.01 | n.s. |
| C20:4n-6       | 1.67 <sup>a</sup>  | 2.19 <sup>a</sup>  | 1.55 <sup>a</sup>                  | 1.71 <sup>a</sup>   | 1.83 <sup>a</sup>                    | 0.11 | n.s. |
| C20:3n-3       | 0.05 <sup>a</sup>  | 0.05 <sup>a</sup>  | 0.05 <sup>a</sup>                  | 0.05 <sup>a</sup>   | 0.06 <sup>a</sup>                    | 0    | n.s. |
| C20:5n-3       | 0.04 <sup>a</sup>  | 0.05 <sup>a</sup>  | 0.04 <sup>a</sup>                  | 0.04 <sup>a</sup>   | 0.04 <sup>a</sup>                    | 0    | n.s. |
| C22:1n-9       | 0.09 <sup>a</sup>  | 0.11 <sup>a</sup>  | 0.11 <sup>a</sup>                  | 0.10 <sup>a</sup>   | 0.11 <sup>a</sup>                    | 0.01 | n.s. |
| C23:0          | 0.04 <sup>a</sup>  | 0.05 <sup>a</sup>  | 0.04 <sup>a</sup>                  | 0.04 <sup>a</sup>   | 0.05 <sup>a</sup>                    | 0    | n.s. |
| C24:0          | 0.03 <sup>a</sup>  | 0.09 <sup>a</sup>  | 0.00 <sup>a</sup>                  | 0.05 <sup>a</sup>   | 0.03 <sup>a</sup>                    | 0.02 | n.s. |
| C22:5n-3       | 0.22 <sup>a</sup>  | 0.29 <sup>a</sup>  | 0.21 <sup>a</sup>                  | 0.23 <sup>a</sup>   | 0.26 <sup>a</sup>                    | 0.02 | n.s. |
| C22:6n-3       | 0.06 <sup>a</sup>  | 0.09 <sup>a</sup>  | 0.05 <sup>a</sup>                  | 0.07 <sup>a</sup>   | 0.07 <sup>a</sup>                    | 0.01 | n.s. |
| ΣSFA           | 33.18 <sup>a</sup> | 34.02 <sup>a</sup> | 35.13 <sup>a</sup>                 | 33.84 <sup>a</sup>  | 34.79 <sup>a</sup>                   | 0.58 | n.s. |
| ΣMUFA          | 40.33 <sup>a</sup> | 37.63 <sup>b</sup> | 38.69 <sup>ab</sup>                | 39.05 <sup>ab</sup> | 38.47 <sup>b</sup>                   | 0.31 | *    |
| ΣPUFA          | 26.49 <sup>a</sup> | 28.35 <sup>a</sup> | 26.18 <sup>a</sup>                 | 27.11 <sup>a</sup>  | 26.74 <sup>a</sup>                   | 0.53 | n.s. |
| PUFA/SFA       | 0.80 <sup>a</sup>  | 0.84 <sup>a</sup>  | 0.75 <sup>a</sup>                  | 0.80 <sup>a</sup>   | 0.77 <sup>a</sup>                    | 0.03 | n.s. |
| Σn-3           | 2.05 <sup>a</sup>  | 2.08 <sup>a</sup>  | 1.99 <sup>a</sup>                  | 2.05 <sup>a</sup>   | 2.01 <sup>a</sup>                    | 0.06 | n.s. |
| Σn-6           | 24.20 <sup>a</sup> | 26.08 <sup>a</sup> | 23.98 <sup>a</sup>                 | 24.83 <sup>a</sup>  | 24.44 <sup>a</sup>                   | 0.48 | n.s. |
| n-6/n-3        | 11.79 <sup>a</sup> | 12.61 <sup>a</sup> | 12.07 <sup>a</sup>                 | 12.18 <sup>a</sup>  | 12.20 <sup>a</sup>                   | 0.17 | n.s. |
| AI             | 0.48 <sup>a</sup>  | 0.50 <sup>a</sup>  | 0.54 <sup>a</sup>                  | 0.49 <sup>a</sup>   | 0.52 <sup>a</sup>                    | 0.02 | n.s. |
| TI             | 0.30 <sup>a</sup>  | 0.30 <sup>a</sup>  | 0.33 <sup>a</sup>                  | 0.30 <sup>a</sup>   | 0.32 <sup>a</sup>                    | 0.01 | n.s. |

<sup>a-c</sup> Mean values in the same row (corresponding to the same parameter) not followed by a common letter differ significantly ( $P < 0.05$ ). Batches: Described in Table 1.SEM: standard error of the mean; Sig.: significance: \* ( $P < 0.05$ ), n.s. (not significant).

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-6 = omega-6; n-3 = omega-3. AI: atherogenic index; TI: thrombogenic index.

2017).

The samples presented an n-6 / n-3 ratio close to 12. These values are close to those obtained by Mancini et al. (2020) when evaluating the lipid profile of raw rabbit meat burgers. Linoleic acid (C18:2n-6) was the

major polyunsaturated fatty acid (PUFA) found in the samples (82.81% of the total PUFA). The PUFA/SFA ratio varied from 0.75 to 0.84 ( $P > 0.05$ ) and the atherogenicity (AI) and thrombogenicity (TI) indexes were close to 0.5 and 0.3, respectively. These values are considered as

desirable for a human diet (Ulbricht & Southgate, 1991; Wood et al., 2004), and indicate an excellent nutritional lipid quality of dry-cured rabbit legs produced in this study.

### 3.5. Volatile compounds

The volatile compounds of the dry-cured rabbit legs are shown in Table 6. More than 50 volatile compounds were detected, of which 30 were grouped according to their probable origin (Ordóñez, Hierro, Bruna, & De La Hoz, 1999), which corresponded to 7 compounds from carbohydrate fermentation, 9 from amino acid degradation, and 14 compounds from lipid oxidation.

The most abundant compounds were derived from carbohydrate fermentation, corresponding to about 40 to 50% of the total peak area. The compounds from lipid oxidation represented the second-largest peak area (32 to 42%), followed by the compounds from amino acid degradation (16 to 20%).

In quantitative terms, acetic acid ( $P > 0.05$ ) was the main compound identified in the samples, representing from 21 to 25% of the total peak area (44 to 63% of compounds from carbohydrate fermentation). It was also identified as a major compound in low-sodium dry-cured meat products by other authors (Corral, Salvador, & Flores, 2013; Dos Santos, Campagnol, Fagundes, Wagner, & Pollonio, 2015) and plays an important role in the sensory quality, as it contributes to the ripened aroma (Marco, Navarro, & Flores, 2007). Acetone ( $P < 0.001$ ) and acetoin ( $P < 0.001$ ) were other compounds derived from carbohydrate fermentation also found in large quantities in the samples. These compounds are also very important, as they provide a meaty flavor (Flores, 2018; García-González, Tena, Aparicio-Ruiz, & Morales, 2008). The compound 2,3-butanediol ( $P < 0.05$ ) was also identified, which has a positive impact on the aroma even in small amounts, as it provides fruity (Ordóñez et al.,

1999) and buttery notes (Montel, Masson, & Talon, 1998). Despite some statistical differences in some minoritary volatile compounds, the sodium reformulation did not affect the majoritary volatile compounds from carbohydrate fermentation. Of the 9 compounds identified from amino acid degradation, 5 were affected by the sodium reformulation. In general, higher 3-methyl-butanal and 2-methyl-butanal concentrations were observed in the reduced-sodium samples ( $P < 0.01$ ). These compounds positively contribute to the sensory quality of dry-cured meat products, as they confer cheese, nutty, and salty notes (Andrés, Cava, & Ruiz, 2002). The compound 3 -methyl thiophene, which contributes to the ripened aroma (Stahnke, Holck, Jensen, Nilsen, & Zanardi, 2002), was also found in greater quantities in the samples with the addition of KCl when compared with the sample Na<sub>100%</sub>. Other authors also reported that the reduction or replacement of NaCl by KCl led to an increase in compounds from the amino acid degradation of dry-cured meat products (Dos Santos, Campagnol, Cavalcanti, et al., 2015). This result may be due to the differences in pH values during the manufacture of dry-cured rabbit legs (Fig. 1b), as also reported by Olivares, Navarro, Salvador, and Flores (2010).

The compounds from the lipid oxidation, such as aldehydes, ketones, and alcohols, have a great impact on odor due to their low olfactory threshold (Ordóñez et al., 1999). Of the 14 compounds identified in the samples of this study, 7 were affected by the sodium reformulation. Hexanal is a compound that confers a rancid aroma and is considered the major marker of lipid oxidation of meat products (Lorenzo, Bermúdez, & Franco, 2013; Pérez-Santaescolástica et al., 2019). In the present study, hexanal was the second major compound from lipid oxidation, representing from 3 to 6% of the total peak area. The samples with 50% NaCl reduction (Na<sub>50%</sub> and Na<sub>50%</sub> G) exhibited the lowest hexanal levels ( $P < 0.001$ ). This result is in agreement with other studies (Dos Santos, Campagnol, Cavalcanti, et al., 2015) and may be due to the lower ionic

**Table 6**  
Volatile compounds (expressed as AU × 10<sup>5</sup>) of low-sodium dry-cured rabbit leg.

|                           | Na <sub>100%</sub> | Na <sub>50%</sub>  | Na <sub>50%</sub> K <sub>50%</sub> | Na <sub>50%</sub> G | Na <sub>50%</sub> K <sub>50%</sub> G | SEM | SIG  |
|---------------------------|--------------------|--------------------|------------------------------------|---------------------|--------------------------------------|-----|------|
| Carbohydrate fermentation |                    |                    |                                    |                     |                                      |     |      |
| Acetoin                   | 3.05 <sup>b</sup>  | 5.37 <sup>b</sup>  | 4.47 <sup>b</sup>                  | 7.25 <sup>a</sup>   | 3.15 <sup>b</sup>                    | 2.9 | ***  |
| 1-Butanol                 | 0.49 <sup>b</sup>  | 0.55 <sup>ab</sup> | 0.62 <sup>a</sup>                  | 0.24 <sup>c</sup>   | 0.51 <sup>b</sup>                    | 0.0 | ***  |
| 2,3-Butanediol            | 0.11 <sup>ab</sup> | 0.11 <sup>ab</sup> | 0.07 <sup>b</sup>                  | 0.61 <sup>a</sup>   | 0.03 <sup>b</sup>                    | 0.1 | *    |
| 3-Methylbutyl acetate     | 0.05 <sup>c</sup>  | 0.10 <sup>b</sup>  | 0.04 <sup>c</sup>                  | 0.33 <sup>a</sup>   | 0.06 <sup>bc</sup>                   | 0.0 | ***  |
| Acetone                   | 4.85 <sup>a</sup>  | 4.55 <sup>a</sup>  | 3.24 <sup>bc</sup>                 | 3.68 <sup>b</sup>   | 3.06 <sup>c</sup>                    | 0.1 | ***  |
| Ethyl Acetate             | 0.43 <sup>a</sup>  | 0.40 <sup>a</sup>  | 0.33 <sup>a</sup>                  | 0.29 <sup>a</sup>   | 0.43 <sup>a</sup>                    | 0.0 | n.s. |
| Acetic acid               | 12.0 <sup>a</sup>  | 10.76 <sup>a</sup> | 9.42 <sup>a</sup>                  | 9.80 <sup>a</sup>   | 12.41 <sup>a</sup>                   | 2.9 | n.s. |
| Amino acid degradation    |                    |                    |                                    |                     |                                      |     |      |
| 3-Methyl thiophene        | 1.48 <sup>bc</sup> | 1.36 <sup>c</sup>  | 1.91 <sup>ab</sup>                 | 1.18 <sup>c</sup>   | 2.25 <sup>a</sup>                    | 0.1 | **   |
| Phenylethyl alcohol       | 0.09 <sup>a</sup>  | 0.29 <sup>a</sup>  | 0.14 <sup>a</sup>                  | 0.19 <sup>a</sup>   | 0.16 <sup>a</sup>                    | 0.0 | n.s. |
| 3-Methyl-1-butanol        | 1.69 <sup>a</sup>  | 1.64 <sup>a</sup>  | 1.63 <sup>a</sup>                  | 1.37 <sup>a</sup>   | 1.65 <sup>a</sup>                    | 0.6 | n.s. |
| 3-Methylbutanal           | 0.49 <sup>b</sup>  | 1.21 <sup>a</sup>  | 0.78 <sup>b</sup>                  | 0.63 <sup>b</sup>   | 1.38 <sup>a</sup>                    | 0.0 | **   |
| 2-Methylbutanal           | 0.31 <sup>d</sup>  | 0.61 <sup>bc</sup> | 0.44 <sup>cd</sup>                 | 0.77 <sup>ab</sup>  | 0.85 <sup>a</sup>                    | 0.0 | **   |
| 3-Methyl butanoic acid    | 3.14 <sup>a</sup>  | 2.51 <sup>a</sup>  | 1.57 <sup>a</sup>                  | 1.80 <sup>a</sup>   | 3.00 <sup>a</sup>                    | 0.5 | n.s. |
| 2-Methyl butanoic acid    | 1.75 <sup>a</sup>  | 1.57 <sup>a</sup>  | 0.85 <sup>a</sup>                  | 1.18 <sup>a</sup>   | 1.92 <sup>a</sup>                    | 0.3 | n.s. |
| Benzeneacetaldehyde       | 0.19 <sup>b</sup>  | 0.24 <sup>b</sup>  | 0.18 <sup>b</sup>                  | 0.74 <sup>a</sup>   | 0.18 <sup>b</sup>                    | 0.0 | **   |
| 2,6-Dimethylpyrazine      | 0.88 <sup>ab</sup> | 1.93 <sup>a</sup>  | 0.06 <sup>b</sup>                  | 0.29 <sup>b</sup>   | 0.29 <sup>b</sup>                    | 0.2 | **   |
| Lipid oxidation           |                    |                    |                                    |                     |                                      |     |      |
| 2-Ethylhexanol            | 1.30 <sup>a</sup>  | 1.13 <sup>a</sup>  | 1.58 <sup>a</sup>                  | 0.75 <sup>a</sup>   | 1.55 <sup>a</sup>                    | 0.4 | n.s. |
| 1-Penten-3-ol             | 1.61 <sup>ab</sup> | 1.56 <sup>ab</sup> | 2.08 <sup>a</sup>                  | 0.84 <sup>b</sup>   | 2.08 <sup>a</sup>                    | 0.2 | *    |
| 2-Methylfuran             | 0.26 <sup>c</sup>  | 0.26 <sup>cd</sup> | 0.39 <sup>a</sup>                  | 0.20 <sup>d</sup>   | 0.53 <sup>a</sup>                    | 0.0 | ***  |
| 2-Pentylfuran             | 0.87 <sup>a</sup>  | 0.86 <sup>a</sup>  | 0.97 <sup>a</sup>                  | 0.84 <sup>a</sup>   | 0.86 <sup>a</sup>                    | 0.1 | n.s. |
| 2-Nonanone                | 0.75 <sup>b</sup>  | 0.85 <sup>a</sup>  | 0.47 <sup>b</sup>                  | 0.85 <sup>b</sup>   | 0.31 <sup>b</sup>                    | 0.1 | **   |
| 2-Octanone                | 0.26 <sup>a</sup>  | 0.42 <sup>a</sup>  | 0.26 <sup>a</sup>                  | 0.39 <sup>a</sup>   | 0.26 <sup>a</sup>                    | 0.0 | n.s. |
| 2-Pentanone               | 0.24 <sup>b</sup>  | 0.24 <sup>b</sup>  | 0.15 <sup>b</sup>                  | 0.79 <sup>a</sup>   | 0.14 <sup>b</sup>                    | 0.0 | ***  |
| 1-Octen-3-ol              | 4.89 <sup>a</sup>  | 4.89 <sup>a</sup>  | 4.63 <sup>a</sup>                  | 3.68 <sup>a</sup>   | 4.33 <sup>a</sup>                    | 1.1 | n.s. |
| 2-Heptanone               | 1.18 <sup>a</sup>  | 1.59 <sup>a</sup>  | 1.30 <sup>a</sup>                  | 1.57 <sup>a</sup>   | 1.58 <sup>a</sup>                    | 0.2 | n.s. |
| Nonanal                   | 0.22 <sup>a</sup>  | 0.09 <sup>ab</sup> | 0.10 <sup>ab</sup>                 | 0.04 <sup>b</sup>   | 0.09 <sup>ab</sup>                   | 0.0 | *    |
| 1-Hexanol                 | 0.63 <sup>a</sup>  | 0.49 <sup>a</sup>  | 0.54 <sup>a</sup>                  | 0.63 <sup>a</sup>   | 0.48 <sup>a</sup>                    | 0.1 | n.s. |
| 1-Pentanol                | 1.11 <sup>b</sup>  | 1.16 <sup>b</sup>  | 1.69 <sup>a</sup>                  | 0.95 <sup>b</sup>   | 1.38 <sup>ab</sup>                   | 0.1 | *    |
| Hexanoic acid             | 2.36 <sup>a</sup>  | 1.15 <sup>a</sup>  | 2.92 <sup>a</sup>                  | 0.90 <sup>a</sup>   | 1.31 <sup>a</sup>                    | 0.7 | n.s. |
| Hexanal                   | 3.16 <sup>a</sup>  | 1.45 <sup>b</sup>  | 2.26 <sup>a</sup>                  | 1.33 <sup>c</sup>   | 2.95 <sup>a</sup>                    | 0.2 | ***  |

<sup>a-d</sup> Mean values in the same row not followed by a common letter differ significantly ( $P < 0.05$ ). Batches: Described in Table 1. SEM: standard error of the mean; Sig.: significance: \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ), \*\*\* ( $P < 0.001$ ), n.s. (not significant).

strength of these treatments as reported by Hernández, Park, and Rhee (2002), who investigated the effect of different NaCl and KCl ratios on the lipid oxidation of pork meat.

### 3.6. Sensory analysis

Because the scarce literature related to the development and, specifically, the study of the sensory properties of a dry-cured rabbit product using the consumer perception, the sorting task technique was used in this research to study the sensory characteristics of this product category. As is widely known, the sorting of dry-cured rabbit samples was converted to Euclidean distances being subsequently represented on a perceptual map by means of the DISTATIS technique. The first two dimensions of the DISTATIS (Fig. 2a) accounting for 56% of the explained variance revealing that the positioning of the samples is explained by the concentration of NaCl as well as the different substitute salts/additives used in the formulation. Products with “100% NaCl” and “50% NaCl + 50% KCl” were positioned on the right side of the first dimension. In the center of this same dimension, was located the treatment with “50% NaCl” (with sodium reduction), while the treatments added of glutamate (“50% NaCl” and “50% NaCl +50% KCl”) were positioned to the left. The positioning of these three samples groups indicates that both the reduction and substitution of NaCl as well as the incorporation of monosodium glutamate impacted the sensory characteristics. To verify any changes in sensory attributes, the textual data analyzed by CA was displayed in the Fig. 2b (Dim1: 54.10% and Dim 2: 25.89%).

The sensory description of the samples revealed that although the samples made with the standard concentration of salt (4%) for this product type, the use of KCl as a substitute for NaCl caused drastic changes in the description of products added of substitute salts. For example, while 100% NaCl samples was characterized as “salty”, the salt-substitute sample were described as “dark”, “hard”, “dry”. This fact shown that substitution impaired mainly the texture dimension of sensory profile of the product.

When going from substitution to reduction of NaCl, the taste dimension was impaired, being perceived the reduced-sodium product as “less salty”. Finally, the incorporation of monosodium glutamate (known umami flavor enhancer) improved the sensory description of the NaCl substituted products being characterized as “salted in right amount”, “sweet”, and “cured”. The dry-cured rabbit formulations with a 50% reduction in sodium did not show any sensory improvement due to the addition of glutamate as they were perceived by consumers as “non-tasty”. These interesting results open the door to new research focusing on meat products with sodium substitution using flavor enhancers with healthier characteristics such as mushroom extracts (Harada-Padermo et al., 2020), among others.

## 4. Conclusion

This study evaluated for the first time the feasibility of using rabbit leg for the manufacture of dry-cured meat products. A sodium reformulation was proposed to give the product a healthier appeal. The samples presented protein contents higher than 30% and all EAA had an amino acid score greater than 1.0. In addition, the samples presented low fat contents (<6%) and a healthy fatty acid profile. The sodium reformulation reduced the sodium content by 46.2% and a healthy Na/K ratio was observed in the samples with the addition of KCl. For all samples, the compounds from carbohydrate fermentation consisted of the major volatile compounds. In addition, the 50% NaCl reduction decreased the hexanal levels. The sensory evaluation showed that the lower saltiness of the samples with 50% NaCl reduction was perceived by consumers, and the addition of KCl negatively affected the texture attributes. However, the addition of MG was effective in reducing these defects and improved the consumers' perception.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.meatsci.2020.108372>.

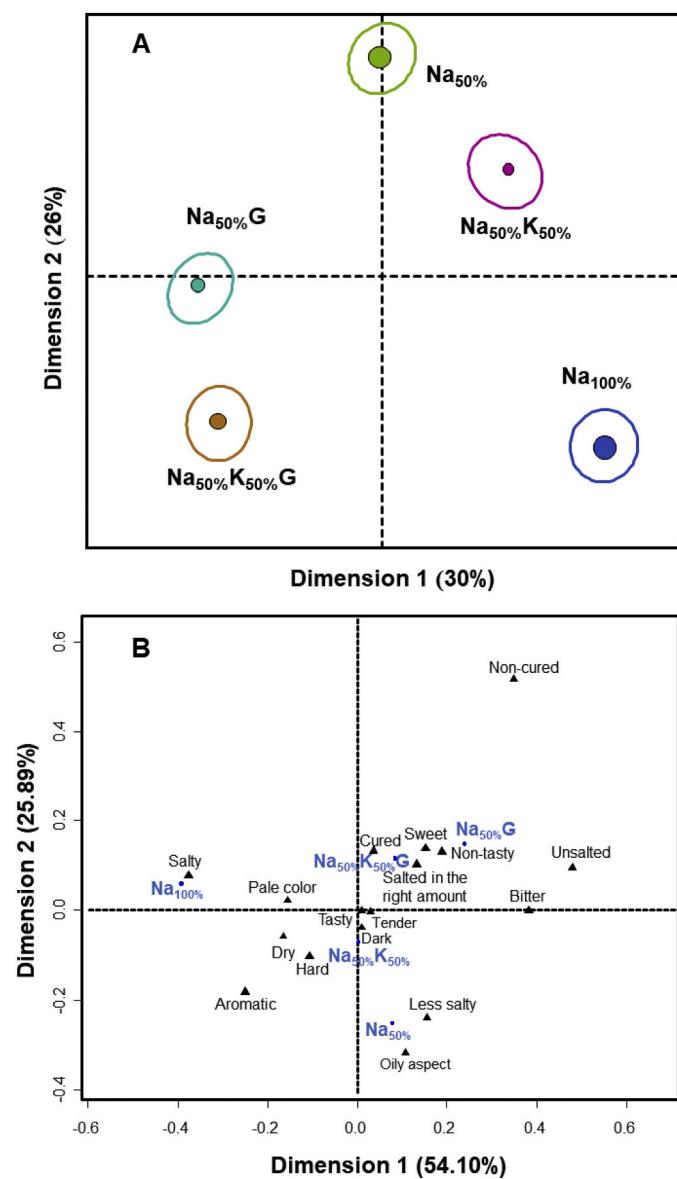


Fig. 2. Sensory map of the dry-cured rabbit samples (A) and attributes (B) evaluated by the sorting task technique.

[org/10.1016/j.meatsci.2020.108372](https://doi.org/10.1016/j.meatsci.2020.108372).

### Declaration of Competing Interest

The author have no conflicts of interest for disclose to paper entitled “Low-sodium dry-cured rabbit leg: a novel meat product with healthier properties”.

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#### **4.2 Artigo 2: Sodium reformulation and its impact on oxidative stability and sensory quality of dry-cured rabbit legs**

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##### **Abstract**

Low-sodium dry-cured rabbit legs were made with a reduction or replacement of 50% of NaCl by KCl and the addition of monosodium glutamate (MG). Oxidative stability was evaluated during 90 days of storage at 20 °C by determining pH, redox potential (Eh), and TBARS. The sensory quality was also assessed through the application of tests with consumers (acceptance and CATA). The oxidative stability of the samples was not affected by the sodium reformulation. The evolution of pH, redox potential, and TBARS proved that all samples can be sold for 90 days at 20 °C. The samples with KCl showed lower scores ( $P < 0.05$ ) for the attribute flavor and liking and were characterized in the CATA test by the attributes "astringent flavor", "bitter taste" and "metallic flavor". The addition of GM to samples with 50% NaCl reduction provided an acceptance and a sensory profile similar to the sample with 100% NaCl.

**Keywords:** exotic meat; KCl; flavor enhancer; lipid oxidation; CATA; shelf life.

## 1 Introduction

The COVID-19 pandemic has changed the lifestyle of many consumers and the search for food to boost immune function is increasingly in evidence (Galanakis, 2020). Thus, the demand for foods with healthier characteristics is expected to increase in the coming years. The meat industry can participate in this niche market; however, it is necessary to innovate and improve the nutritional quality of meat products.

The use of raw materials that have nutritional characteristics beneficial to human health is a way of producing healthier meat products. In this sense, rabbit meat is an excellent alternative, as it contains very low fat and cholesterol levels and high contents of proteins and essential amino acids. It is also rich in phosphorus, selenium, and B vitamins (Dalle Zotte & Szendrő, 2011; Hermida et al., 2006).

Ripening is another strategy to provide healthier characteristics to meat products since proteolysis caused by the action of endogenous or exogenous enzymes can lead to the formation of bioactive peptides (Toldrá et al., 2020). The main biological activities identified in peptides derived from ripened meat products include the positive effect on blood pressure and the improvement of the immune system (Escudero et al., 2012; Lorenzo et al., 2018).

NaCl reduction is another approach to produce healthier meat products, reducing the sodium intake and therefore the risk of developing high blood pressure. Thus, the risk of death from cardiovascular disease or COVID-19 is reduced (He et al., 2020; Fang et al., 2020). The substitution of NaCl for KCl is one of the most effective strategies to reduce the sodium content of meat products (Pateiro et al., 2021), and the great advantage of this approach is the antimicrobial effect of KCl similar to NaCl (Bidlas and Lambert, 2008). However, KCl can impair the sensory quality of the product, thus the combination with flavor enhancers may be an interesting strategy (Inguglia et al., 2017). In addition, KCl can also affect the oxidative

stability during the shelf life of the product, which depends on the KCl concentration used in the formulation and the type of meat product (dos Santos et al., 2017; Vidal et al., 2019).

In a recent study, our research group studied rabbit meat to produce a cured meat product with healthier characteristics. The strategy was the manufacture a dry-cured rabbit leg with a reduction or replacement of 50% of NaCl by KCl and the addition of monosodium glutamate (MG) as a flavor enhancer. In addition to the low-sodium profile, the products exhibited protein contents greater than 30% and an amino acid score greater than 1.0 for all essential amino acids. In addition, a low fat content (<6%) and healthy lipid indexes were obtained, and oleic acid was the major fatty acid (> 30% of the total fatty acids). The sorting task was used to determine the consumers' perception of this novel product, and the results suggested that KCl reduced saltiness while MG reduced the sensory defects caused by the addition of KCl (Pedro et al., 2021). However, despite its healthy profile, for the production on a commercial scale, the product should be subjected to a broader sensory evaluation, and the oxidative stability must be evaluated during the storage to determine its shelf life. Thus, in this study, the sensory quality of low-sodium dry-cured rabbit legs was assessed through the application of acceptance tests and Check-All-That-Apply (CATA) questionnaire, and the oxidative stability was evaluated during 90 days of storage at 20 °C through the determination of pH values, redox potential (Eh), and TBARS values.

## 2 Materials and methods

### 2.1 Obtaining the raw material and manufacture of dry-cured legs

The raw material used in this study was obtained from sixty-five New Zealand White rabbits (*Oryctolagus cuniculus*) raised under the same rearing system. The animals (160 days old) were slaughtered in a slaughterhouse with municipal inspection according to the procedures required by the Brazilian legislation (BRASIL, 2000). For appropriate development of *rigor mortis*, the carcasses were placed in a cold chamber with a temperature of  $3 \pm 1$  °C at

the end of the slaughter. The pH values 2 hours post-mortem were  $5.68 \pm 0.08$ , which is within the normal pH range (BOU et al., 2020). The legs were removed from the carcasses after 12 hours of cooling. The legs were manually massaged in running water to remove blood residues that can compromise the quality of the product. In the end, the legs were hung for 3 minutes for dripping, vacuum-packed individually, and stored for 30 days at -18 °C.

The legs were thawed for 72 hours at  $3 \pm 1$  °C and relative humidity of 85-90%. They had an average weight of  $257.84 \pm 21.15$ g. Five legs were used to characterize the raw material ( $67.89 \pm 1.84\%$  moisture;  $22.47 \pm 0.21\%$  protein;  $2.61 \pm 0.50\%$  fat) (AOAC, 2006). The remaining legs were randomly divided into 5 treatments. The control (Na<sub>100%</sub>) was made with 4% NaCl, which is within the salt concentration range used in similar products (Ventanas et al., 2005). For the other treatments, a reduction or replacement of 50% of NaCl by KCl and the addition of MG was performed as follows: Na<sub>50%</sub>: 2% NaCl; Na<sub>50%</sub>K<sub>50%</sub>: 2% NaCl + 2% KCl; Na<sub>50%</sub>G: 2% NaCl + 0.3% MG; Na<sub>50%</sub>K<sub>50%</sub>G: 2% NaCl + 2% KCl + 0.3% MG. In addition, 100 ppm NaNO<sub>2</sub> and 200 ppm NaNO<sub>3</sub> were used as curing agents in all treatments. The manufacturing process was performed as described by Pedro et al. (2021). The quantities of the ingredients were calculated according to the weight of each leg, and the incorporation was performed by manual massage. After salting, the legs were hung and stored in a curing chamber under the following conditions:  $2 \pm 1$  °C and  $90 \pm 5\%$  RH from day 1 to 7; and  $12 \pm 1$  °C and  $70 \pm 5\%$  RH from day 8 to 28 of storage. After completion of the manufacturing process, the cured legs were individually vacuum-packed and stored at  $20 \pm 1$  °C for 90 days. The results of water activity, weight loss, proximate composition (moisture, protein, fat), sodium and potassium contents, fatty acid profile, amino acid profile, and volatile compounds of cured legs were published in a previous study (Pedro et al., 2021).

## 2.2 Sampling

*Biceps femoris* (BF) muscle was removed from cured legs after 1, 30, 60, and 90 days of storage and used for the instrumental measurements and sensory evaluation. The determinations of pH, redox potential (Eh), and TBARS values were performed in triplicate on days 1, 30, 60, and 90 of storage using three legs per treatment. The sensory evaluation was performed shortly after the completion of the manufacturing process using 8 legs per treatment.

## 2.3 Determination of pH, redox potential, and TBARS

The oxidative changes in the dry-cured legs were monitored during the 90 days of storage by determining the pH, Eh, and TBARS values. For that, 5 g of ground BF was homogenized with 50 mL of distilled water, and pH and Eh were determined in the mixture using a digital dual channel pH meter equipped with a diffusion and platinum electrode (DM-23-DC, Digimed, Brazil). TBARS were determined as described by Bruna et al. (2001), and the results were expressed in milligrams of malonaldehyde per kg of sample.

## 2.4 Sensory evaluation

One hundred regular consumers (at least once a week) of meat products and adults (18 to 60 years old) participated in the sensory evaluation. The BF samples were manually cut into slices (~1 mm thick) and served to consumers (5g) in white plastic cups encoded with 3 digits at ~8 °C. The samples were served in sequential monadic form, following a Williams' Latin square design (Macfie et al., 1989). Mineral water and crackers were served for all consumers to cleanse the palate between samples.

First, consumers performed the acceptance test using a nine-point hedonic scale (1: disliked very much; 9: liked very much) to evaluate the parameters color, aroma, taste, texture, and liking. Afterward, consumers were asked to complete a Check-all-that-apply (CATA) questionnaire containing the descriptors related to the sensory characteristics of the products. Before the CATA test, consumers were instructed to select the descriptors considered

appropriate to characterize the samples, with no limitations. The descriptors were defined in a previous study (Pedro et al., 2021) as follows: appearance attributes (ideal color, pale color, pleasant color), aroma (cured aroma, pleasant aroma, rancid aroma), flavor (acid taste, astringent taste, bitter taste, metallic taste, pleasant flavor, rancid flavor, sweet taste, unpleasant taste), texture (dry, juicy, hard, pleasant texture, soft) and NaCl level (little salt, ideal salt, very salty).

The sensory evaluation was performed in individual booths, with light and temperature control. The study protocol was approved by the Research Ethics Committee of the Federal University of Santa Maria (CAAE 16218519.0.0000.5346) and all participants signed informed consent to participate in the research.

## 2.5 Statistical analysis

### 2.5.1 Instrumental data

Instrumental data were analyzed using a generalized linear model (ANOVA). The variables “treatments” and “storage time”, and the interaction “treatments \* storage time” were considered as a fixed effect, and each rabbit leg ( $n = 3$ ) was considered as a random effect. The Tukey’s test at a 5% level of significance ( $P < 0.05$ ) was used to compare the means between treatments.

### 2.5.2 Sensory data

A mixed linear model was used to analyze the results of the acceptance test, using the treatments as a fixed effect, and consumers as a random effect. Tukey’s test was used to compare the means ( $P < 0.05$ ). The results of the CATA test were analyzed in binary format (0- unmarked attribute; 1- marked attribute) and the Cochran Q test was applied to compare the samples independently for each descriptor. Correspondence analysis was performed using the chi-square distance calculated from a frequency table, in which the rows corresponded to the treatments, and the columns corresponded to the CATA descriptors. The principal component

analysis was applied to assess the correlation between the CATA descriptors (tetrachoric correlation) and the overall acceptance scores (biserial correlation).

### **3 Results and Discussion**

#### **3.1 pH**

The pH values were affected by the interaction between the treatments and the storage time ( $P < 0.001$ ). The pH of the treatments varied from 6.06 to 6.5 during the 90 days of storage (Figure 1). An increase in pH values was observed after the 60<sup>th</sup> day of storage, which may be due to the release of alkaline compounds produced during the proteolytic reactions (Campagnol et al., 2011). The pH behavior observed in the present study was expected, once a similar trend has been reported for other types of dry-cured products (Sara et al. 2014; Bermúdez et al., 2014). The samples with 50% NaCl reduction (Na<sub>50%</sub> and Na<sub>50%</sub>G) showed higher pH values when compared with the control (Na<sub>100%</sub>) on days 60 and 90 of storage. A similar result was also observed for the samples containing KCl (Na<sub>50%</sub>K<sub>50%</sub> and Na<sub>50%</sub>K<sub>50%</sub>G) at the end of storage (day 90). Blanco et al (1997) reported that NaCl concentration between 5% and 6% completely inhibited the action of protease enzymes (Calpain and Cathepsin D). Thus, probably, the higher NaCl concentration of the control (Na<sub>100%</sub>) greatly reduced the proteolytic activity of the enzymes, leading to less release of free amino acids with basic character (Toldrá, 1992). In agreement with the present results, Tomažin et al. (2020) and Škrlep et al. (2016) found a higher proteolytic activity in low-sodium dry-cured ham. Santos et al. (2015) also reported similar results for fermented sausages made with 50% NaCl reduction.

#### **3.2 Eh**

The redox potential was affected by the interaction between the treatments and the storage time ( $P < 0.001$ ). The Eh values ranged from 78.0 to 150.3 during 90 days of storage (Figure 2). In general, Eh values increased during storage, especially after the 60<sup>th</sup> day. This result demonstrates that the samples underwent oxidative reactions during the storage (Heck et

al., 2020). The samples with salt reduction (Na<sub>50%</sub> and Na<sub>50%</sub>G) or substitution (Na<sub>50%</sub>K<sub>50%</sub> and Na<sub>50%</sub>K<sub>50%</sub>G) for KCl showed lower ( $P < 0.001$ ) Eh values when compared with Na<sub>100%</sub> on days 30 and 60 of storage, which suggests that the sodium reformulation reduced the rate of the oxidation reactions (Sena Vaz Leães et al., 2020).

### **3.3 TBARS**

A significant interaction ( $P < 0.001$ ) was found between the treatments and the storage time for TBARS values (Figure 3). The samples showed TBARS values lower than 0.1 mg MDA/kg at the beginning of storage (day 1). As expected, TBARS increased in all samples throughout the storage due to the oxidation of unsaturated fatty acids present in the samples (Pedro et al., 2021). At the end of storage (day 90), the TBARS values ranged from 1.1 to 1.5 mg MDA/kg. The evolution of TBARS values in the present study was similar to reported in the literature for dry-cured meat products (Lloret et al., 2016), can be considered normal. No significant difference in TBARS values was observed for the low-sodium samples analyzed on the same day of storage when compared to Na<sub>100%</sub>. This result is in agreement with other authors, who reported that the addition of up to 6% NaCl provided a very small pro-oxidant effect in dry-cured meat products (Andrés et al., 2004). In addition, Ripollés et al. (2011) also reported that the replacement of NaCl by KCl did not affect the lipid oxidation of dry-cured hams.

### **3.4 Acceptance tests**

The results of the acceptance test are shown in Figure 4. No significant differences ( $P > 0.05$ ) were observed for the attributes color, aroma, and texture of the low-sodium samples when compared with the control Na<sub>100%</sub>. In addition, the taste and liking scores did not differ ( $P > 0.05$ ) between the samples with 50% salt reduction (Na<sub>50%</sub> and Na<sub>50%</sub>G) and Na<sub>100%</sub>. On the other hand, the formulations with 50% replacement of NaCl by KCl received lower ( $P < 0.05$ ) scores for these attributes when compared with Na<sub>100%</sub>. This result was expected, once the

reduction in the sensory quality of products containing high KCl levels is well documented in the literature for other types of meat products, such as fermented (Campagnol et al., 2011; 2012), emulsified (dos Santos Alves et al., 2017; da Silva et al., 2020) and dehydrated (Vidal et al., 2019) products. The addition of MG was not sufficient to suppress the sensory defects caused by the addition of KCl in dry-cured rabbit legs. Opposite results were observed by dos Santos et al. (2014), who reported a positive effect of MG on the sensory quality of fermented sausages made with high KCl levels. This behavior summarizes the difficulty of reducing sodium in the meat industry, and highlights the importance of studies on the different types of meat products, once the differences in the raw material and manufacturing process can affect the results.

### **3.5 Check-all-that-apply (CATA)**

The citation frequency of attributes identified by consumers in the CATA test is shown in Table 1. All 22 descriptors evaluated were identified by at least 10% of consumers. The descriptors "ideal color", "pleasant aroma", "pleasant flavor", "juicy", "pleasant texture", "soft" and "ideal salt" were identified by more than 50% of consumers, therefore showing the great importance of these attributes for the characterization of the sensory profile of dry-cured rabbit leg. The formulation Na<sub>100%</sub> received a higher number of citations for the descriptors "pleasant taste" (50) and "juiciness" (51). The formulations Na<sub>50%</sub> and Na<sub>50%</sub>G were more cited than the others for the descriptors "ideal color" (62 and 70 citations, respectively) and "pleasant aroma" (47 and 50 citations, respectively). In addition, the sample Na<sub>50%</sub>G was more cited for the descriptors "pleasant texture" (56), "soft" (68), and "ideal salt" (60) when compared with the other treatments. When comparing the citations of the samples with a 50% salt reduction, there was a decrease of 14 citations for the descriptor "low salt" and an increase of 15 citations for the descriptor "ideal salt" for the sample Na<sub>50%</sub>G when compared with Na<sub>50%</sub>. This result suggests that a group of consumers identified that MG enhanced the salty taste of the product.

In addition, less than 20% of consumers were able to perceive the negative attributes related to KCl, such as "astringent flavor", "bitter taste", and "metallic taste".

The correspondence analysis used to analyze data from the CATA questionnaire explained 82.24% of the total variance in the first two dimensions (Figure 5). The first dimension (F1: 55.01%) explained the largest percentage, separating the samples into two distinct groups. The formulations Na<sub>100%</sub>, Na<sub>50%</sub>, and Na<sub>50%</sub>G were located in the negative quadrant, while the formulations with KCl (Na<sub>50%</sub>K<sub>50%</sub> and Na<sub>50%</sub>K<sub>50%</sub>G) were located in the positive quadrant of F1, and were characterized by descriptors commonly reported in meat products containing KCl (Santos Alves et al., 2017, da Silva et al, 2020), such as "astringent flavor", "metallic taste", "unpleasant taste", and "bitter taste". The sample Na<sub>50%</sub>G was very close to the Na<sub>100%</sub> in the sensory map, which suggests they have a very similar sensory profile. In addition to being characterized by positive attributes of texture and flavor, such as "pleasant taste", "juicy", "soft" and "pleasant texture", the samples Na<sub>100%</sub>, and Na<sub>50%</sub>G were very close to the descriptor "ideal salt". On the other hand, the sample Na<sub>50%</sub> was close to the descriptor "little salt". Thus, the present results demonstrate that although MG was effective to improve the saltiness of the samples with 50% salt reduction of NaCl, it was not able to suppress the sensory defects caused by the addition of KCl. Similar findings were reported by Pedro et al. (2021), who used the sorting task technique and also reported that the MG increased the saltiness sensation of low-sodium dry-cured rabbit legs.

### **3.6 Correlation between CATA descriptors and the liking scores**

A principal component analysis (PCA) was performed to assess the correlation between the CATA descriptors and the liking scores for the dry-cured rabbit legs (Figure 6). The first two dimensions of the PCA explained 58.17% of the total variance and the scree plot indicated that these dimensions were sufficient to interpret the relationship between the CATA descriptors and the liking scores. The CATA descriptors with a greater positive correlation with the liking

scores were "pleasant taste" (0.374), "juicy" (0.362), "ideal salt" (0.348), "pleasant texture" (0.28) and "cured aroma" (0.203). In contrast, the descriptors "unpleasant taste" (-0.433), "rancid taste" (-0.306), "unpleasant color" (-0.287), and "bitter taste" (-0.239) showed a greater negative correlation with the liking scores.

#### **4 Conclusion**

The behavior of pH, Eh, and TBARS values throughout the 90 days of storage demonstrated that the sodium reformulation proposed in this study did not affect the oxidative stability of dry-cured rabbit legs. The TBARS values were within the range considered acceptable for this type of meat product. Thus, considering that lipid oxidation is the main factor responsible for the deterioration of this type of meat product, the shelf life can be estimated in at least 90 days at 20 °C for all formulations studied. The results of the sensory acceptance and CATA tests showed that the replacement of 50% NaCl by KCl impaired the sensory quality of the product. In addition, these tests indicated that the sample with 50% NaCl reduction and addition of MG had an acceptance and a sensory profile very similar to the sample made with NaCl concentration commonly used in this type of meat product. The present results showed that although the MG was efficient to compensate for the decrease in saltiness caused by the NaCl reduction, it was not effective to suppress the sensory defects caused by KCl.

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## Figure Captions

**Fig. 1.** Overall effects of the treatments and storage time on the pH values of the of low-sodium dry-cured rabbit legs.

<sup>a-e</sup> Averages followed by the same letter did not show any significant difference ( $P > 0.05$ ) by Tukey test. Batches: Na<sub>100%</sub>: 4% NaCl; Na<sub>50%</sub>: 2% NaCl; Na<sub>50%</sub>K<sub>50%</sub>: 2% NaCl and 2% de KCl; Na<sub>50%</sub>G: 2% NaCl and 0.3% monosodium glutamate (MG); Na<sub>50%</sub>K<sub>50%</sub>G: 2% NaCl, 2% KCl and 0.3% MG.

SEM (Standard error of the mean): 0.03

**Fig. 2.** Overall effects of the treatments and storage time on the Eh values of the of low-sodium dry-cured rabbit legs.

<sup>a-e</sup> Averages followed by the same letter did not show any significant difference ( $P > 0.05$ ) by Tukey test. Batches: described in Fig. 1.

SEM (Standard error of the mean): 3.5

**Fig. 3.** Overall effects of the treatments and storage time on the TBARS values of the of low-sodium dry-cured rabbit legs.

<sup>a-h</sup> Averages followed by the same letter did not show any significant difference ( $P > 0.05$ ) by Tukey test. Batches: described in Fig. 1.

SEM (Standard error of the mean): 0.07

**Fig. 4.** Results of the acceptance test of the of low-sodium dry-cured rabbit legs.

<sup>a-b</sup> Averages followed by the same letter did not show any significant difference ( $P > 0.05$ ) by Tukey test. Batches: described in Fig. 1.

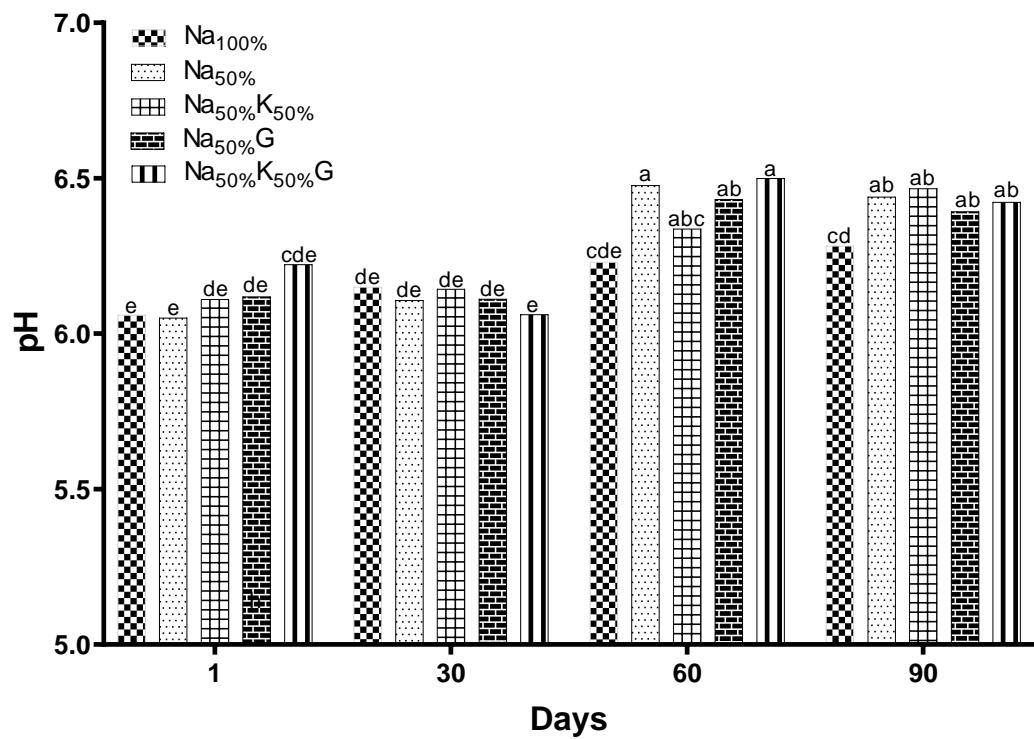
SEM (Standard error of the mean): Color: 0.13; Aroma: 0.14; Taste: 0.17; Texture: 0.13; Liking: 0.13.

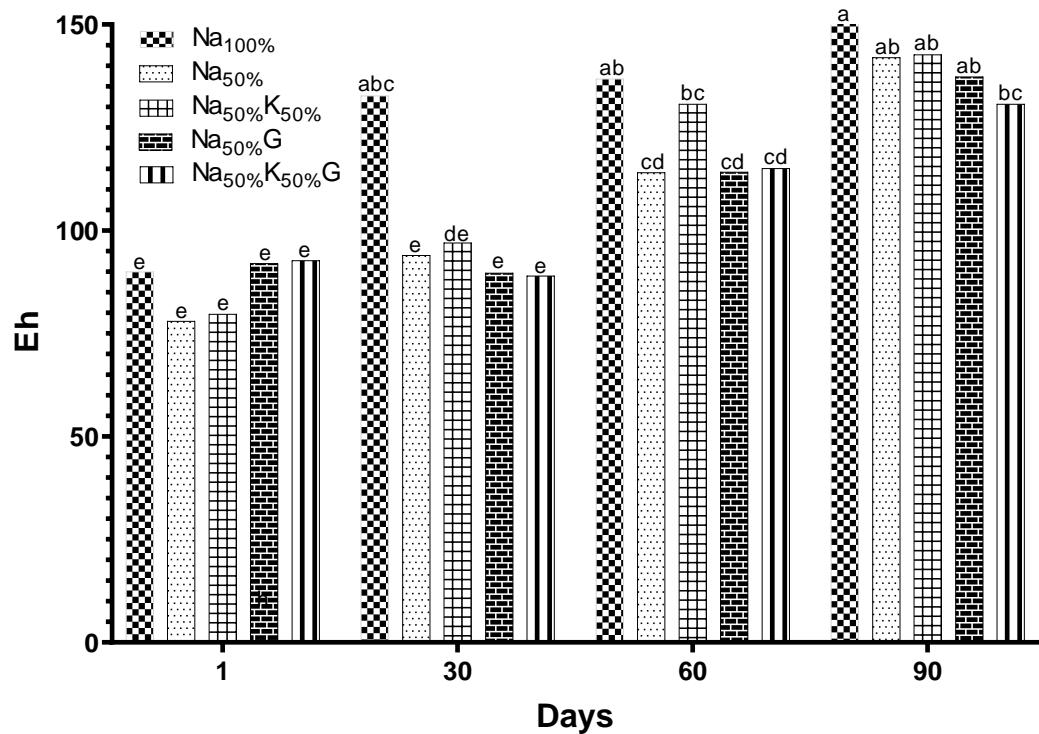
**Fig. 5.** Results of the CATA test of the of low-sodium dry-cured rabbit legs.

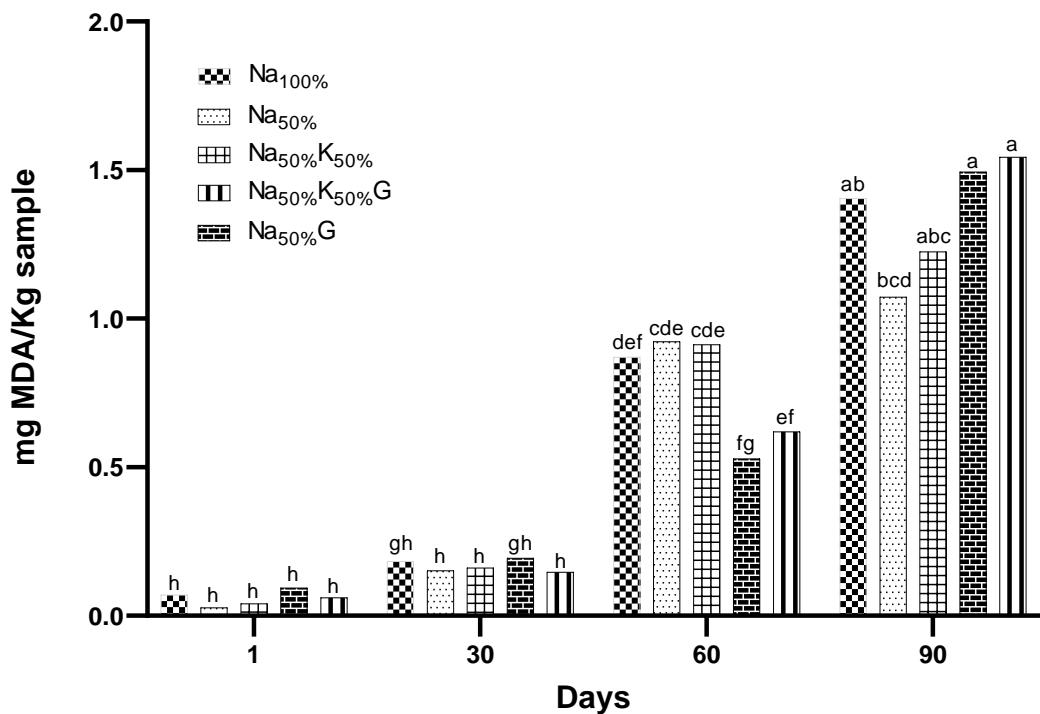
Batches: described in Fig. 1.

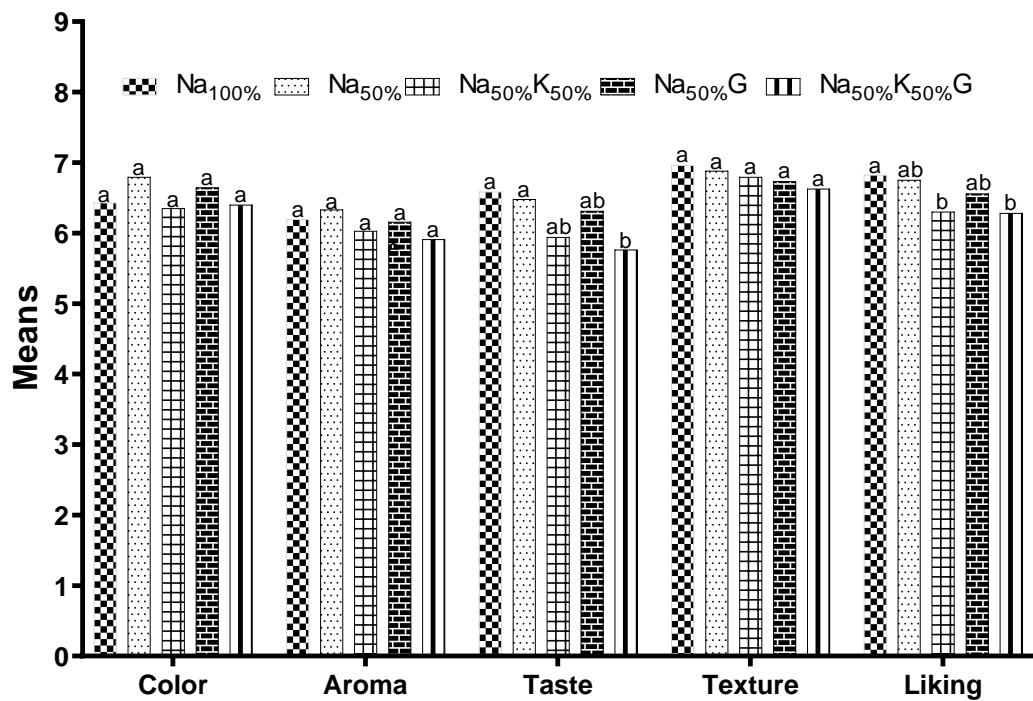
**Fig. 6.** Principal component analysis performed to assess the correlation between the CATA descriptors and the liking scores for the dry-cured rabbit legs.

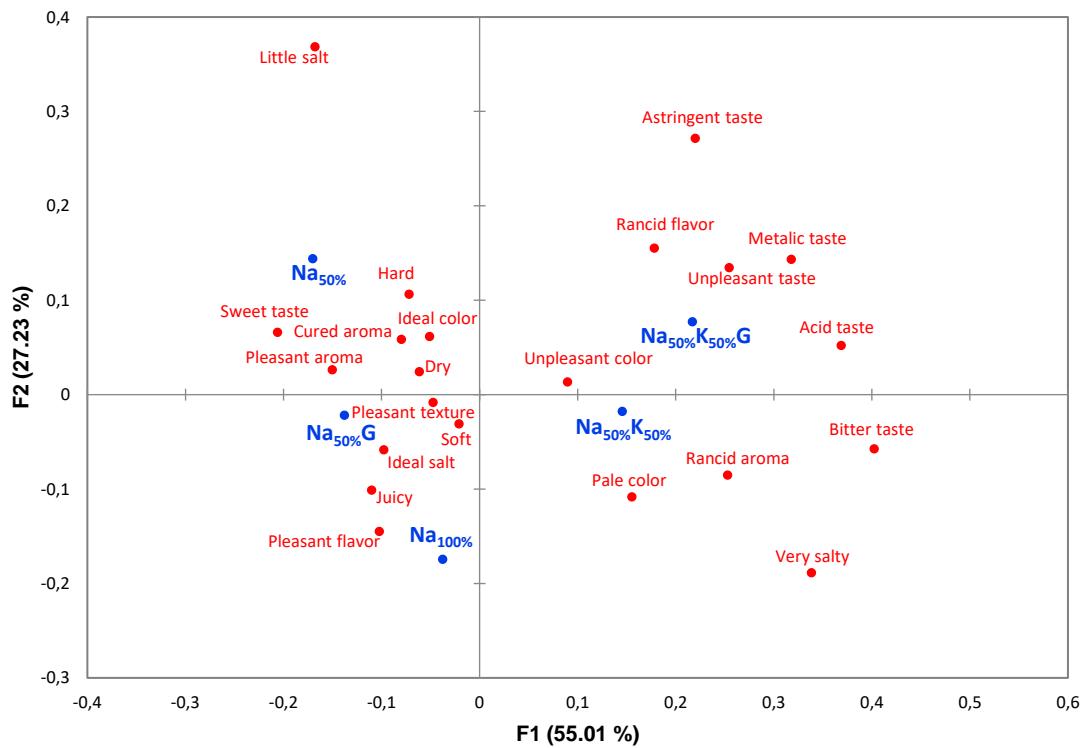
Batches: described in Fig. 1.

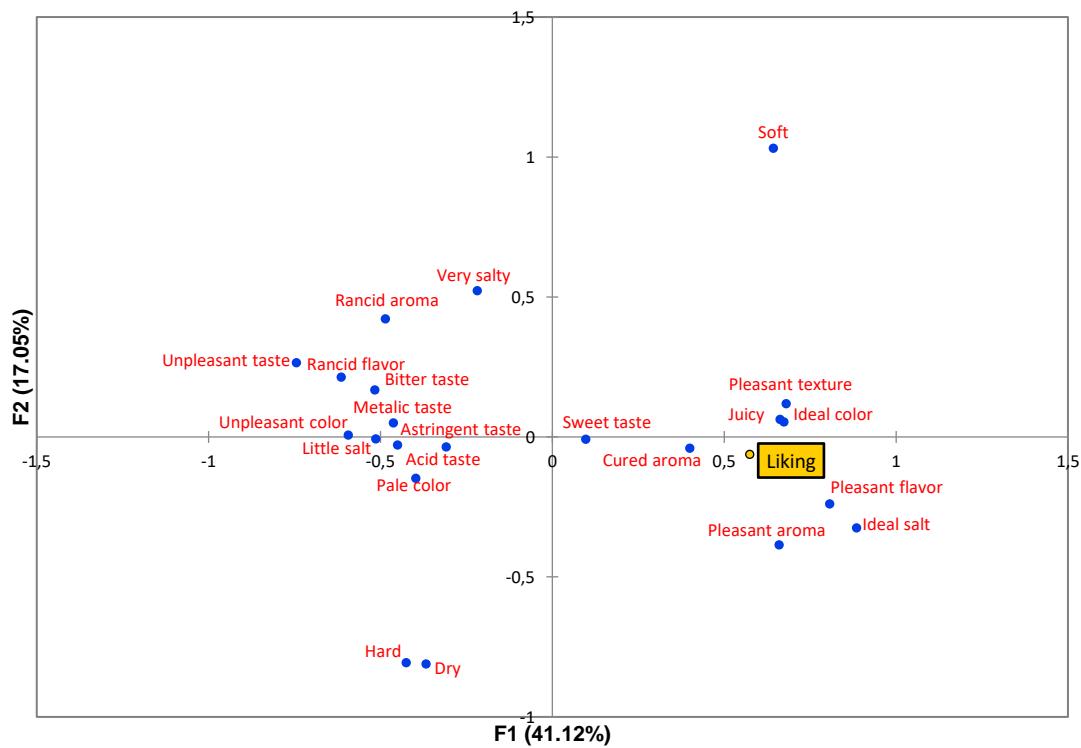
**Fig. 1**

**Fig. 2**

**Fig. 3**

**Fig. 4**

**Fig. 5**

**Fig. 6**

**Table 1.** Frequency of attributes mentioned in the CATA test (n = 100)

| Attributes   |                  | Na100% | Na50% | Na50%K50% | Na50%G | Na50%K50%G |
|--------------|------------------|--------|-------|-----------|--------|------------|
| Color        | Ideal color      | 51     | 62    | 51        | 70     | 57         |
|              | Pale color       | 30     | 18    | 27        | 21     | 28         |
|              | Pleasant color   | 17     | 18    | 23        | 14     | 16         |
| Aroma        | Cured aroma      | 23     | 29    | 24        | 33     | 24         |
|              | Pleasant aroma   | 41     | 47    | 30        | 50     | 34         |
|              | Rancid aroma     | 16     | 8     | 15        | 13     | 20         |
| Taste        | Acid taste       | 7      | 5     | 10        | 7      | 14         |
|              | Astringent taste | 7      | 13    | 11        | 6      | 16         |
|              | Bitter taste     | 12     | 5     | 14        | 9      | 19         |
|              | Metallic taste   | 6      | 7     | 10        | 6      | 13         |
|              | Pleasant flavor  | 50     | 35    | 34        | 49     | 30         |
|              | Rancid flavor    | 8      | 13    | 18        | 15     | 18         |
|              | Sweet taste      | 8      | 12    | 9         | 13     | 6          |
|              | Unpleasant taste | 10     | 12    | 17        | 15     | 23         |
| Texture      | Dry              | 17     | 18    | 12        | 13     | 14         |
|              | Juicy            | 51     | 42    | 34        | 40     | 29         |
|              | Hard             | 6      | 10    | 10        | 13     | 8          |
|              | Pleasant texture | 53     | 51    | 42        | 56     | 48         |
|              | Soft             | 65     | 59    | 58        | 68     | 57         |
| Salt content | Little salt      | 9      | 25    | 9         | 11     | 14         |
|              | Ideal salt       | 51     | 45    | 40        | 60     | 39         |
|              | Very salty       | 19     | 7     | 21        | 9      | 18         |

Batches: Na100%: 4% NaCl; Na50%: 2% NaCl; Na50%K50%: 2% NaCl and 2% de KCl; Na50%G: 2% NaCl and 0.3% monosodium glutamate (MG); Na50%K50%G: 2% NaCl, 2% KCl and 0.3% MG.

## 5 DISCUSSÃO GERAL

O presente estudo elaborou de forma inédita um produto curado a partir de pernis de coelho. Os pernis tiveram seu processo de fabricação encerrado aos 28 dias, momento em que a Aw atingiu valores iguais ou menores que 0,90 (BRASIL, 2000). A perda de peso ficou próxima à 50%, diferindo de outros trabalhos com presunto suíno (RIVAS-CAÑEDO et al., 2020; COLL-BRASAS et al., 2021). Esse alto valor foi atribuído ao baixo percentual de gordura, ao alto teor de umidade a menor tamanho dos pernis.

Foi possível elaborar o produto com baixos percentuais de sódio, atendendo por critérios mais saudáveis como já visto em outros trabalhos com a produção de presunto cru e similares (ALIÑO et al., 2010; ZHANG et al., 2020). Todas as amostras apresentaram um valor proteico superior a 30% e percentuais de gordura menores que 6%, ou seja, esses resultados demonstram as ótimas características nutricionais do produto, sendo superiores aos encontrados em presunto suíno (CAMPOS et al., 2020; BETIOL et al., 2020).

O uso do KCl resultou numa relação Na/K menor que 1,0 que é recomendado para reduzir o risco de surgimento de doenças cardiovasculares (WHO, 2003) e mesmo as amostras dos tratamentos que não utilizaram o KCl tiveram quase 50% de redução na mesma relação, provando serem mais saudáveis. Tanto o uso de GM quanto o uso de KCl, não afetaram a composição dos aminoácidos presentes nas amostras, somado a isso o escore químico de todos os aminoácidos essenciais foi maior do que 1,0, superando a recomendação para adultos da FAO/WHO/UNU (OMS, 2007). Já para os ácidos graxos presentes na composição das amostras, não ocorreram grandes mudanças no perfil, tanto com a redução quanto com a substituição de NaCl. O ácido oleico foi o ácido graxo mais abundante, alcançando praticamente 1/3 dos ácidos graxos totais. Isto demonstra a qualidade nutricional do produto, pois o consumo regular de ácido oleico auxilia no controle dos níveis de colesterol (SMITH et al., 2020), participa na prevenção do câncer de pâncreas (BANIM et al., 2018) e da obesidade (JAGANNATHAN et al., 2020). Em tempos atuais com intensos cuidados sobre os fatores predisponentes a quadros mais graves de infecções por coronavírus, torna-se importante controlar esses pontos, visto que, a obesidade está ligada ao surgimento de um quadro clínico mais grave em pacientes infectados, inclusive levando a óbito (YU et al., 2021).

Foram identificados mais de 50 compostos voláteis, que foram divididos em três grupos de acordo com sua origem, sendo os que se formaram a partir da fermentação dos carboidratos os mais numerosos, seguidos pelos compostos da oxidação lipídica e por fim os da degradação dos aminoácidos. O ácido acético foi o mais abundante e já foi apontado em outros estudos

como um composto importante em produtos cárneos com baixo teor de sódio (DOS SANTOS et al., 2015) e por contribuir com o aroma de maturado desse tipo de produto (MARCO et al., 2009).

Em relação aos resultados da análise sensorial, tanto a substituição e a redução do NaCl, quanto o uso do GM, tiveram efeitos sobre as características sensoriais. Principalmente as características de textura foram afetadas nas amostras com KCl, as quais foram descritas como “dura”, “seca” e “escura”. Já com a diminuição dos níveis de NaCl o sabor foi o mais afetado, sendo descrito como “menos salgado”. Já a adição de GM no tratamento com redução de NaCl aumentou a salinidade, porém o mesmo comportamento não foi observado nas amostras com KCl, as quais foram descritas como “não saborosas”.

Durante a etapa de armazenamento, foram avaliados a evolução dos valores de pH, Eh e TBARS durante 90 dias a  $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$  com o intuito de se avaliar a estabilidade oxidativa dos produtos. Os valores do pH das amostras dos diferentes tratamentos oscilaram entre 6,06 e 6,50 durante os 90 dias de armazenamento e após 60 dias houve um aumento nos valores encontrados. Este fato pode ser atribuído a liberação de compostos alcalinos produzidos em virtude de reações proteolíticas (CAMPAGNOL et al., 2011). Essa mesma tendência pode ser considerada normal e foi observada em outros tipos de produtos curados (SARA et al., 2014; BERMÚDEZ et al., 2014). As amostras com teor de NaCl reduzido nos 60 e 90 dias de armazenamento e as amostras com o uso do KCl nos 90 dias apresentaram maiores valores de pH. O NaCl possui um papel inibidor da atividade das enzimas proteolíticas, consequentemente, houve uma menor liberação de aminoácidos livres básicos (TOLDRÁ, 1992). Concordando com estes resultados, outros estudos com produtos similares encontraram o mesmo comportamento (TOMAŽIN et al., 2020; ŠKRLEP et al., 2016; DOS SANTOS et al., 2015).

Os valores de Eh variaram entre 78,0 e 150,3 durante os 90 dias de armazenamento (Figura 2). Em geral, os valores de Eh aumentaram durante o armazenamento, especialmente à partir do 60º dia. Este resultado demonstra que as amostras sofreram reações oxidativas durante o seu armazenamento (HECK et al., 2020).

Os valores de TBARS aumentaram em todas as amostras durante o armazenamento devido a oxidação dos ácidos graxos insaturados presentes nas amostras. Após 90 dias de armazenamento os valores ficaram entre 1,1 e 1,5 mg MDA/Kg. Valores semelhantes foram reportados em outro estudo com produto similar (LLORET et al., 2016). Ao se analisar o mesmo dia de armazenamento, pode-se observar que os valores de TBARS das amostras reformuladas não diferiram significativamente do controle ( $\text{Na}_{100\%}$ ).

As análises sensoriais do produto durante o armazenamento, foram o teste de aceitação e o Check-all-that-apply (CATA). No teste de aceitação as notas dos atributos cor, aroma e textura não diferiram ( $P > 0,05$ ) entre Na<sub>100%</sub> e as amostras reformuladas. As notas de sabor e aceitação global também não diferiram ( $P > 0,05$ ) entre as amostras com redução de 50% de NaCl (Na<sub>50%</sub> e Na<sub>50%G</sub>) e Na<sub>100%</sub>. Por outro lado, as amostras com substituição de 50% de NaCl por KCl receberam notas menores ( $P < 0,05$ ) nos atributos de sabor e aceitação global que Na<sub>100%</sub>. Fato já relatado em outros estudos com produtos similares (CAMPAGNOL et al., 2011; 2012; DOS SANTOS et al., 2017; VIDAL et al., 2019; DA SILVA et al., 2020). A adição GM não conseguiu compensar os defeitos sensoriais causados pela adição de KCl e o resultado obtido está em desacordo com os encontrados por Dos Santos et al. (2014). Quanto aos resultados do teste CATA todos os 22 descritores avaliados foram assinalados por pelo menos 10% dos consumidores. A amostra Na<sub>50%G</sub> foi a mais citada no descritor “aroma agradável”, “textura agradável”, “macia” e “sal ideal”. Houve um aumento de 15 citações no descritor “sal ideal” na amostra Na<sub>50%G</sub> em relação a Na<sub>50%</sub>. Este resultado sugere que um grupo de consumidores identificou que o GM realçou o sabor salgado do produto. Além disso, mais de 80% dos consumidores não identificaram os atributos negativos ligados ao uso de KCl. A amostra Na<sub>50%G</sub> ficou muito perto no mapa sensorial de Na<sub>100%</sub>, o que sugere que essas amostras possuem um perfil sensorial muito similar.

## 6 CONCLUSÃO GERAL

Os resultados obtidos comprovam que todos os pernis curados apresentaram baixos teores de gordura e altos percentuais de proteína, demonstrando a alta qualidade do produto final. Porém, a perda de peso foi próxima a 50%, o que reduz o rendimento ou aproveitamento da carne. Esse fato provavelmente ocorreu em função do alto teor de umidade, baixa quantidade de gordura e também ao tamanho reduzido dos pernis. Esse é um ponto que pode ser melhor abordado nos próximos trabalhos, com a possibilidade de adição de algum tipo de proteção superficial para reduzir a perda de umidade do produto e consequentemente a quebra.

O tratamento com redução de 50% de NaCl apresentou redução de 46,2% do teor de sódio em relação a Na<sub>100%</sub>. Porém, apenas o tratamento com KCl teve uma taxa de Na/K abaixo de 1,0, que é um valor adequado para reduzir o risco do surgimento de doenças cardiovasculares.

A substituição de 50% de NaCl por KCl e a adição de GM não alteraram o perfil de ácido graxos. O ácido oleico foi o ácido graxo mais abundante encontrado nas amostras e isto é benéfico nutricionalmente, pois esse ácido graxo quando consumido regularmente pode reduzir os riscos de ocorrência de arterosclerose. As amostras dos pernis curados de todos os tratamentos apresentaram uma relação PUFA/SFA variando de 0,75 a 0,84 e índices de aterogenicidade (AI) e trombogenicidade (TI) próximos a 0,5 e 0,3, respectivamente, sendo esses valores considerados muito saudáveis.

Alguns compostos voláteis foram afetados com a substituição parcial do sódio, porém o impacto não foi evidente, demonstrando a necessidade de mais estudos para esclarecer esses efeitos. Dos 14 compostos identificados nesse estudo, 50% deles foram afetados pela reformulação sódica. O hexanal, que é um composto que imprimiu um aroma rançoso em produtos cárneos curados, foi o segundo principal composto da oxidação lipídica encontrado chegando a representar entre 3 e 6% da área total do pico, ou seja, esse ponto pode ser melhor explorado em estudos futuros com estratégias que busquem reduzir os níveis de hexanal.

Os resultados obtidos demonstraram que é possível aumentar o consumo da carne de coelho através da elaboração do presunto curado com baixo teor de sódio, sendo mais saudável e com grande valor nutricional sem afetar negativamente a maioria das propriedades avaliadas.

Quanto a estabilidade oxidativa dos pernis durante 90 dias de armazenamento, a mesma não foi prejudicada em decorrência da reformulação sódica, fato demonstrado pela evolução nos valores de pH, Eh e TBARS. Os valores de TBARS ficaram dentro da faixa considerada

aceitável para este tipo de produto cárneo, podendo-se concluir que o shelf life a 20 °C de todas as amostras é de no mínimo 90 dias.

Os resultados dos testes de aceitação e CATA mostraram que a substituição de 50% de NaCl por KCl prejudicou a qualidade sensorial do produto. Além disso, estes testes indicaram que a amostra com 50% de redução de NaCl e adição de GM teve uma aceitação e um perfil sensorial muito similar a amostra elaborada com o teor de NaCl comumente utilizado neste tipo de produto cárneo. Estes resultados demonstraram, portanto, que o GM foi eficiente para compensar a diminuição da salinidade causada pela redução de NaCl, mas que não foi capaz de compensar os defeitos sensoriais causados pelo KCl. Os resultados contraditórios quanto ao uso do GM no teste de aceitação sugerem a necessidade de mais estudos quanto a sua utilização, pela grande diversidade de produtos cárneos e dos seus diferentes processos de fabricação.

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