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DEBORAH SILVA DO AMARAL

EFEITO DA INCORPORAÇÃO DE QUITOSANA E QUITOSANA MODIFICADA NA
QUALIDADE, FUNCIONALIDADE TECNOLÓGICA E VIDA DE PRATELEIRA DE
SALSICHAS COM REDUZIDO TEOR DE GORDURA

JOÃO PESSOA - PB

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Orientadora: Dr^a Marta Suely Madruga

Co-orientadoras: Dr^a Maria Manuela Estevez Pintado

Dr^a Alejandra Cardelle-Cobas

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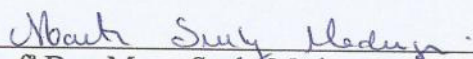
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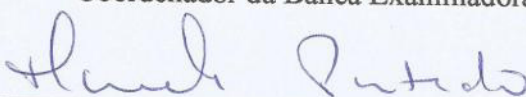
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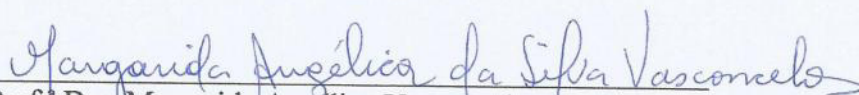
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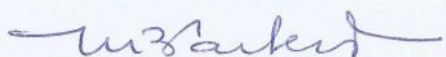
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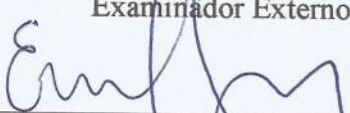

Profª Dra. Marta Suely Madruga – PPGCTA/ UFPB
Coordenador da Banca Examinadora

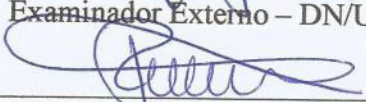

Profª Dra. Maria Manuela E. Pintado – ESB/UCP
Co-Orientador

Profª Dra. Alejandra Cardelle-Cobas – ESB/UCP
Co-Orientador


Prof.ª Dra. Margarida Angelica Vasconcelos - DN/UFPE
Examinador Externo


Profª Dra. Maria Teresa Bertoldo Pacheco - ITAL/SP
Examinador Externo


Prof. Dr. Evandro Leite de Souza - DN/UFPB
Examinador Externo – DN/UFPB


Profª Dra. Rita de Cassia Queiroga - PPGCTA/UFPB
Examinador Interno

Deus, pela benção da vida e pelo amor incondicional.

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*“Só existe uma coisa bela, suave, atraente, útil, luminosa: o
que Deus quer de ti no momento presente”.*

Chiara Lubich

RESUMO

O objetivo deste estudo foi avaliar a inclusão da quitosana e quitosana modificada (quitosana-glicose), em concentração adequada para atender a alegação de saúde, em salsichas com reduzido teor de gordura, sobre a funcionalidade tecnológica e a vida de prateleira destas salsichas. Para isso, um primeiro estudo foi realizado, no qual a quitosana (2 % m/m) foi adicionada em salsichas elaboradas com carne suína e três níveis de gordura (5%, 12,5% e 20%, m/m). As salsichas foram submetidas ao armazenamento refrigerado a 4 ± 1 °C por 15 dias e avaliadas em relação aos parâmetros microbiológicos, físico-químicos e sensoriais. Posteriormente, considerando a inexistência de estudos sobre a incorporação de quitosana em produtos cárneos caprino, realizou-se um segundo estudo, semelhante ao anterior, para avaliar o efeito da incorporação da quitosana (2 % m/m) em salsicha elaborada com carne caprina e similares níveis de gordura na qualidade microbiológica e físico-química durante o armazenamento refrigerado (4 ± 1 °C) por 15 dias. Por fim, no terceiro estudo foi avaliado o efeito do derivado da quitosana (2% m/m), comparando com a quitosana base (2% m/m), em salsicha elaborada com carne caprina e reduzido teor de gordura (10% m/m). As salsichas foram armazenadas a 4 ± 1 °C por 21 dias, e avaliadas nos parâmetros microbiológicos, físico-químicos e sensoriais. Nos três estudos uma formulação sem adição dos polímeros (quitosana e quitosana modificada) foi usada como controle. Os resultados indicaram que o uso de 2% (m / m) de quitosana e seu derivado (correspondendo a 1 g de quitosana por salsicha de 50g cada em uma porção de 150g) para garantir a ingestão de 3 g de quitosana por dia é tecnologicamente viável para uso na elaboração de salsicha com carne de porco e cabra. Além do potencial funcional, a quitosana e o derivado da quitosana proporcionaram aumento da estabilidade das salsichas tratadas, uma vez que provocaram a redução do crescimento microbiológico quando comparadas a amostra controle. Adicionalmente, proporcionaram uma melhoria na cor vermelha, resultando em efeitos positivos na aparência, uma emulsão mais estável, pela maior capacidade de ligar água e gordura, e uma textura mais firme por aumentar as forças de compressão, sem afetar negativamente as propriedades sensoriais das salsichas. A quitosana e a quitosana modificada mostraram comportamento semelhante, mas o uso da quitosana resultou em maior atividade antioxidante do que o seu derivado, considerando a maior redução dos valores de TBARS e maior teor de ácidos graxos insaturados e poli-insaturados. Entretanto, o derivado da quitosana promoveu maior cor vermelha (a^*) e menor dureza que a quitosana. Portanto, os resultados da utilização da quitosana na fabricação de salsichas caprina, a qual foi estudada pela primeira vez, indicaram que a quitosana e a quitosana modificada apresentaram potencial interessante para serem utilizados na elaboração de produtos cárneos, uma vez que podem proporcionar maior qualidade e vida de prateleira, agregando valor à matéria-prima de baixo valor comercial, além de permitir a obtenção de um produto funcional com alegação de saúde e redução de gordura.

Palavras-chave: quitosana, quitosana modificada, quitosana-glicose, produto cárneo funcional, redução de gordura, vida de prateleira

ABSTRACT

The aim of this study was to evaluate the inclusion of chitosan and modified chitosan (chitosan-glucose) in appropriate concentration to meet the health claim in sausages with reduced fat, on the technological functionality and shelf life of these sausages. For this, an initial study was conducted, in which chitosan (2% w/w) was added to sausages prepared with pork and three levels of fat (5%, 12.5% and 20% w/w). The sausages were submitted to cold storage at 4 ± 1 °C for 15 days and evaluated in terms of microbiological, physico-chemical and sensory parameters. Subsequently, considering the absence of studies on incorporation of chitosan in meat goat products, carried out a second study, similar to the above to evaluate the effect of incorporation of chitosan (2% w/w) in sausage made with goat meat, and similar levels of fat in the microbiological and physico-chemical quality during cold storage (4 ± 1 °C) for 15 days. Finally, in the third study was evaluated the effect of the derivative of chitosan (2% w/w) in comparison with chitosan base (2% w/w) in sausage made with goat meat and reduced fat content (10% w/w). The sausages were stored at 4 ± 1 °C for 21 days and evaluated by microbiological, physico-chemical and sensory parameters. In all three studies a formulation without the addition of polymers (chitosan and modified chitosan) was used as control. The results indicated that the use of 2% (w / w) chitosan and its derivative (corresponding to 1 g of chitosan per sausage of 50g each in a portion of 150g) to ensure ingestion of 3 g of chitosan per day is technologically feasible for use in the preparation of sausage with pork and goat meat. In addition to the functional potential, chitosan and chitosan derivative provided increased stability of the treated sausages since it caused the reduction of microbial growth when compared to the control sample. Additionally, they provided an improvement in red color, resulting in positive effects on the appearance, a more stable emulsion for the greater ability to bind water and fat, and a firmer texture to increase the compression forces, without negatively affecting the sensory properties of sausages. The chitosan and modified chitosan showed similar behavior, but the use of chitosan resulted in higher antioxidant activity than its derivative, considering further reduction of TBARS values and increased content of unsaturated fatty acids and polyunsaturated. However, the chitosan derivative promoted a greater red color (a^*) and lower hardness than chitosan. Therefore, the results of the use of chitosan in the manufacture of goat sausages, which was studied for the first time, indicated that the chitosan and modified chitosan showed potential interest to be used in the preparation of meat products, since they can provide higher quality and shelf life, adding value to the raw material of low commercial value, besides allow to obtain of a functional product with the health claim and fat reduction.

Keywords: chitosan, modified chitosan, chitosan-glucose, functional meat product, fat reduction, shelf life

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ABREVIACES

ANOVA	Anlise de Varincia
AOAC	Association of Official Analytical Chemists
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CAPES	Coordenao de Aperfeioamento de Pessoal de Nvel Superior
CIE	Commision Internationale de Lclairage
CFU	Colony Forming Units
DD	Degree of Deacetylation
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
FCT	Fundao para a Cincia e a Tecnologia
Glc	Glucose
HMWC	High Molecular Weight Chitosan
LMWC	Low Molecular Weight Chitosan
LMWC-Glc	LMWC-Glc derivative obtained by Maillard reaction
MMWC	Medium Molecular Weight Chitosan
MW	Molecular Weight
MDA	Malondialdehyde
NO-Mb	Nitrosomyoglobin
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
RTE	Ready-to-eat
TBA	2-Thiobarbituric acid
TBARS	Thiobarbituric Acid Reactive Substances
TBHQ	Tert-butylhydroquinone
TC	Total Cholesterol
TG	Triglycerides
TPA	Texture Profile Analysis
VRBGA	Violet Red Bile Glucose Agar
WRC	Water Retention Capacity

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1. INTRODUÇÃO

A quitosana é um polímero linear de β -1,4-glucosamina, não tóxico, obtido por desacetilação da quitina, de ocorrência natural e que está presente no exoesqueleto de crustáceos, tais como caranguejo, camarão, lagosta e lagostim, ou na estrutura de fungos, sendo considerado o segundo polissacarídeo mais abundante na natureza depois da celulose (BRUGNEROTTO et al. 2001; CHATTERJEE et al., 2005; LIU et al., 2006).

A aplicação da quitosana em alguns campos é limitada pela sua baixa solubilidade em valores de pH perto da neutralidade ou alcalinos, e em alguns casos, pelo seu elevado peso molecular, resultando em elevada viscosidade em água (TIKHONOV et al., 2006). Com isso, diversas pesquisas têm indicado que este polissacarídeo com vários pesos moleculares e com graus de desacetilação pode ser modificado através de processos químicos para melhorar sua solubilidade, capacidade de absorção de água e gordura ou propriedades bioativas, o que aumentaria significativamente sua aplicação (HAFDANI; SADEGHINIA, 2011; SILVA; SANTOS; FERREIRA, 2006). Entre as potenciais propriedades ativas melhoradas com a modificação, poderiam estar as propriedades prebióticas, considerando que a redução dos grupos amino poderiam converter a quitosana em forma fermentável pelas bactérias intestinais, podendo ainda melhorar as suas propriedades anti-inflamatórias.

Diferentes tipos de quitosana têm atraído considerável interesse devido às suas propriedades biológicas, tais como, antimicrobiana (BENTO et al. 2011; LI et al., 2010; MAHAE; CHALAT; MUHAMUD, 2011; ORTEGA-ORTIZ et al., 2010; RODRÍGUEZ-NÚÑEZ et al., 2012; XIA et al. 2010), antioxidante (FERNANDES et al., 2010; MAHAE; CHALAT; MUHAMUD, 2011), capacidade de ligar água e gordura (SAYAS-BARBERÁ et al. 2011), emulsionante (CHEVALIER et al., 2001), anti-inflamatória (FERNANDES et al., 2010) e antitumoral, (VINSOVÁ; VAVRÍKOVÁ, 2011), as quais são interessantes para diversas áreas de aplicações na indústria de alimentos (XIA et al., 2010).

A quitosana também tem sido descrita como hipocolesterolêmica em numerosos estudos *in vitro* (ANRAKU et al., 2011, LIU; ZHANG; XIA, 2008) e *in vivo* em animais (XIA et al., 2010, YAO; HUANG; CHIANG, 2008) e em humanos (GALLAGHER et al. 2002). Assim, devido às evidências consistentes sobre a capacidade de quitosana para diminuir o colesterol sérico, a Autoridade Europeia para a Segurança dos Alimentos (EFSA) aprovou uma alegação de saúde que estabelece que "o consumo regular de quitosana contribui para a manutenção dos níveis normais de colesterol no sangue". No Brasil, a

Agência Nacional de Vigilância Sanitária (ANVISA) diz que “A quitosana auxilia na redução da absorção de gordura e colesterol. Seu consumo deve estar associado a uma alimentação equilibrada e hábitos de vida saudáveis”. A fim de suportar o pedido, a EFSA e a ANVISA exigem uma quantidade nos alimentos de pelo menos 3 g / dia de quitosana (ou derivados de quitosana) em uma ou mais porções (EFSA, 2011; ANVISA, 2008).

Em produtos alimentícios, a quitosana oferece uma vasta gama de aplicações, incluindo a preservação de alimentos da deterioração microbiana, a formação de filmes comestíveis-biodegradáveis, a coagulação das proteínas e lipídios de águas residuais, aumento de gelificação em surimi e produtos da pesca e clarificação/desacidificação de sucos de frutas (ZEMPLICA, 2013). Alguns estudos também reportam a redução de gordura em produtos cárneos pela incorporação de quitosana (KIM; THOMAS, 2007, LIN; CHAO, 2001). Um dos primeiros estudos foi realizado por Lin e Chao (2001), os quais mostraram que a quitosana pode ser usada de forma positiva em uma salsicha de estilo chinês com reduzido teor de gordura.

Salsicha é um produto cárneo popular, tradicionalmente constituído de carne picada, água, ligantes, gordura e temperos (ESSIEN, 2003). Este tipo de produto apresenta algumas percepções negativas devido ao alto teor de gordura que habitualmente o caracteriza, podendo contribuir para uma diminuição em seu consumo e consequente perda de quota de mercado, especialmente quando as considerações de saúde são um importante critério de qualidade (JIMÉNEZ-COLMENERO et al. 2010). Outro aspeto negativo habitualmente associado com este produto fresco é o frequente uso de aditivos sintéticos para evitar reações de oxidação lipídica prejudiciais, controlar o crescimento de contaminantes e patógenos alimentares e consequentemente aumentar a vida de prateleira (GEORGANTELIS et al. 2007). Assim, a reformulação destes produtos com base em estratégias de processamento é uma tendência importante para desenvolver alimentos que promovam a melhoria da saúde dos consumidores (JIMÉNEZ-COLMENERO et al., 2010).

Atualmente, têm crescido a busca por produtos naturais como alternativa de conservantes em alimentos, visto que a segurança dos aditivos sintéticos tem sido questionada nos últimos anos (OLIVEIRA et al., 2012). A este respeito, a quitosana tem atraído muito interesse como alternativa natural, devido à sua comprovada segurança (MAHAE; CHALAT; MUHAMUD, 2011). Nos Estados Unidos, em 2001, a FDA certificou a quitosana com um produto “GRAS – Geralmente Reconhecido como Seguro”.

Estudos comprovam o potencial tecnológico da quitosana adicionada em salsichas elaboradas com carne suína (AMARAL et al., 2015; GEORGANTELIS et al., 2007; LIN; CHAO, 2001; SOULTOS et al, 2008), entretanto o uso da quitosana em concentração adequada para atender a alegação de saúde recomendada pela EFSA, no que respeita à diminuição de colesterol, são escassos. Da mesma forma, os efeitos da incorporação da quitosana em produtos derivados da caprinocultura ainda não foram estudados. A carne de porco é uma das mais tradicionais e populares consumida no mundo, destacando-se por ser rica em proteína e pobre em carboidratos, com níveis semelhantes de colesterol ou gordura saturada comparada com a carne bovina, ave e ovelha, além de apresentar conteúdos de ácidos graxos oléico e linoleico diferenciado (TEIXEIRA; RODRIGUES, 2013).

A carne caprina tem um grande potencial de mercado por causa de seu valor nutricional, como baixo teor de gordura, alta digestibilidade, elevado teor de proteína, ferro e ácidos graxos insaturados, quando comparada a outras carnes vermelhas (MADRUGA, 2004). Em geral, os melhores cortes caprinos – Lombo, paleta, perna - são vendidos a preços elevados, enquanto os cortes restantes têm baixa aceitação do consumidor e baixo valor comercial (NASSU et al., 2003). Webb et al. (2005) enfatizaram que o baixo apelo da carne caprina para alguns consumidores pode ser devido a textura mais ressecada e dura, particularmente na carne de animais mais velhos. Neste contexto, a industrialização de cortes menos nobre e da carne de animais mais velhos, pode ser uma alternativa para agregar valor à carne *in natura* de baixa atratividade comercial pelo desenvolvimento de novos produtos com propriedades sensoriais melhoradas e maior vida de prateleira (MADRUGA; BRESSAN, 2011).

Diante do exposto, considerando a inexistência de estudos sobre a incorporação de quitosana e quitosana modificada em produtos derivados de carne caprina, bem como estudos que refletem a potencialidade da quitosana e seus derivados como um ingrediente funcional em produtos cárneos, o objetivo deste estudo foi de incluir quitosana e quitosana modificada em concentração adequada para atender as recomendações da EFSA, e assegurar alegação de saúde, em uma matriz cárnea com redução de gordura e avaliar os efeitos na qualidade, estabilidade e vida de prateleira. Assim, salsichas foram produzidas inicialmente com carne suína e posteriormente com carne caprina, em diferentes teores de gordura (5, 10, 12,5 e 20%), seguida de armazenamento sob refrigeração a $4 \pm 1^{\circ}\text{C}$ e avaliação de seus parâmetros microbiológicos, físico-químicos e sensoriais.

2. REVISÃO DE LITERATURA

ARTIGO 1

USE OF CHITOSAN TO IMPROVE QUALITY AND FUNCTIONALITY OF MEAT PRODUCTS: A REVIEW*

Submetido na revista Critical Reviews in Food Science and Nutrition
(ANEXO A)

**USE OF CHITOSAN TO IMPROVE QUALITY AND FUNCTIONALITY OF MEAT
PRODUCTS: A REVIEW**

**Deborah S. do Amaral^a, Alejandra Cardelle-Cobas^b, Marta S. Madruga^a, Maria
Manuela E. Pintado^{b*}**

^aDEA - Department of Food Engineering, Technology Centre, Federal University of Paraiba,
58051-900 João Pessoa, Paraiba, Brazil

^bCBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior
de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital,
Apartado 2511, 4202-401 Porto, Portugal

*Corresponding author:

E-mail address: mpintado@porto.ucp.pt

Tel.: +351 225580097

Fax: +351-225090351

Abstract

The consumers are more aware of the link between diet and health. Thus, the meat products presents some negative perceptions, due to high fat content and artificial preservatives. In this sense, chitosan has attracted especial interest from the industry as a potential natural food preservative since it exhibits strong antimicrobial activity and antioxidant. In addition to the preservation effect, different studies reported the possibility of a fat reduction in meat products by the incorporation of chitosan with no significant impact on sensory characteristics and textural properties. Although, in the context of a healthy diet, it is necessary to conduct further studies, the addition of chitosan it is well documented and proved that it can act positively on the quality and shelf-life of food and specifically, in meat and meat products, without offering risk for consumer health and without adversely affecting sensory characteristics. Therefore, the use of chitosan can result in functional healthy products technologically feasible.

Keywords: functional food, antioxidant, antimicrobial, fat reduction

1. Introduction

During the last years, the advance on the knowledge between diet and health has caused that consumers are becoming more and more aware of the necessity of change their habits towards the consumption of more healthy products (Grasso et al., 2014). The meat products are a kind of products which presents some negative perceptions, most of them related to its high fat content (Lin & Chao, 2001) and the artificial preservatives which usually present (Oliveira et al., 2012). These negative aspects linked to the fact that their consumption has increased in the last five years has caused that the Health World Organization gave, last year, the advice about the risks that their consumption could be in the long term.

Thus, in order to satisfy the demand for safe and high quality meat and meat products researchers and industry have joined efforts to improve the products. In this sense, many of the negative connotations associated with processed meat products could be overcome by reducing the unhealthy constituents (such as saturated fats, salt and nitrates) and simultaneously incorporating bioactive ingredients with health-promoting efficacy (Grasso et al., 2014). In this sense, “healthier” meats products may better respond to consumers interested in preventing health risks associated with the consumption of processed meat products (Grasso et al., 2014).

In relation to safety and stability during storage, the meat products are prone to spoilage by both microbial contamination and lipid oxidation due, mainly, to their high fat content, the perishable nature of the raw materials and in some cases, due to the lack of thermal processing (Georgantelis et al., 2007). With the aim of ensuring an extension of the shelf life of these kind of products, various synthetic additives such as nitrites, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) (Georgantelis et al., 2007; Mariutti et al., 2011; Oliveira et al., 2012) are widely

used in the food industry (Jayasena & Jo, 2013). However, their use has been restricted because of possible health risks and toxicity (Jayasena & Jo, 2013; Oliveira et al., 2012). A fact that has contributed to an increase in consumer concerns toward healthier meat and meat products and a demand for natural food additives over the years (Jayasena & Jo, 2013). Thus, most of the recent researches have been comprising studies on new preservative compounds from natural sources, such as grains, oilseeds, spices, fruit and vegetables (Chen et al. 1996), as well as, different plant parts like leaves, roots, stems, fruits, seeds and bark (Shah et al., 2014) or from byproducts from the food industry. Among these different natural compounds, chitosan has attracted especial interest from the meat industry as a potential natural food preservative due to its important functional activities (Shahidi et al., 1999)

Thus, several studies have demonstrated the antimicrobial effects of chitosan, applied either individually or in combination with other natural antimicrobials such as essential oils, in meat systems over the past few years (Kanatt et al., 2008b, 2013). Some studies have reported that chitosan could be used effectively to inhibit microbial spoilage and delaying lipid oxidation at certain concentrations in meat products (Soultos et al., 2008; Georgantelis et al. 2007; Roller et al. 2002; Lin & Chao 2001, Amaral et al. 2015).

In addition to the preservation effect exerted by the incorporation of chitosan in meat, some researches have also found concerning other beneficial effects. Thus, different studies recorded the possibility of a fat reduction in meat products by the incorporation of chitosan with no significant impact on sensory characteristics and textural properties (Amaral et al. 2015; Kim & Thomas, 2007; Lin & Chao, 2001).

Besides all these beneficial aspects demonstrated in meat products, chitosan has been proved to be non-toxic, biodegradable and biocompatible (Kanatt et al., 2013). Soultos et al. (2008) reported that chitosan has been Generally Recognised as Safe (GRAS) by the United States Food and Drug Administration (U.S. FDA) in 2001. In Japan, chitosan has been

approved as a food additive since 1983 (Vasilatos & Savvaidis, 2013) and, in Europe, the European Food Safety Authority (EFSA) approved a health claim which establishes that “regular consumption of chitosan contributes to the maintenance of normal blood cholesterol concentrations” (EFSA, 2011).

Although the high potential of chitosan has been extensively reported its application to food industry is still scarce. Thus, the objective of this paper is to compile and critically analyse all the relevant information concerning the benefits of chitosan when applied in food matrices, particularly in meat products.

2. Chitosan: structure, production and modification

Chitin (fig 1.) is a linear polymer of N-acetyl glucosamine units linked by $\alpha(1\rightarrow4)$ bonds, after cellulose, is the most abundant polysaccharide in nature, and is the primary structural component of the shells of crustaceans, arthropods and fungal cell wall and is obtained mainly as a byproduct of the fishing industry (Hafdani & Sadeghinia, 2011). Partial deacetylation of chitin gives rise to chitosan, a polysaccharide composed of units of glucosamine (2-amino-2-deoxy-D-glucose) and N-acetyl glucosamine (2-acetamido-2-deoxy-D-glucose) linked by $\beta(1\rightarrow4)$ bonds. (Brugnerotto et al. 2001; Chatterjee et al., 2005; Tikhonov et al., 2006). The objective of the deacetylation is to obtain a more versatile polymer (Domard, 2011). Chitosan is the only natural polysaccharide that presents cationic character due to the amino group, which at low pH is protonated and can interact with negatively charged compounds such as proteins, anionic polysaccharides (alginates, carragenates, pectins, etc. ..) fatty acids, bile acids and phospholipids (Agulló *et al.*, 2003; Guo *et al.*, 2005). This behavior along with its biocompatibility and biodegradability, as well as it being free from toxicity, has led to the chitosan being used in such diverse fields as

technology, food, cosmetics, medicine, biotechnology, agriculture and the paper industry (Tharananthan and Kittur, 2003; Kim and Rajapakse 2005; Kurita 2006).

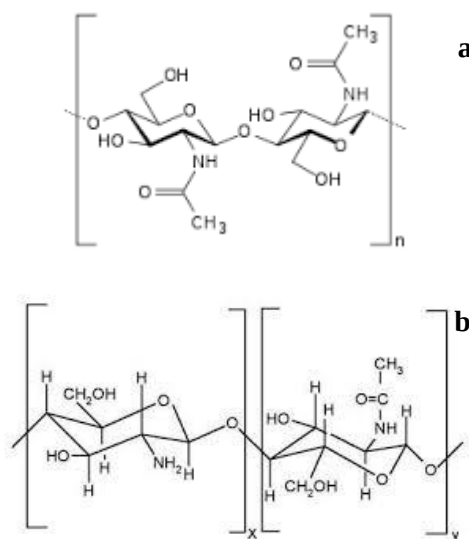


Fig 1. Chemical structure of chitin (a) and chitosan (b)

The main physicochemical characteristics of chitosan are determined by its degree of deacetylation (DD) and its molecular weight (MW). These factors influence the functional and biological activities of chitosan, such as viscosity, solubility and antimicrobial activity among other bioactive properties (Liu et al., 2006; Hafdani & Sadeghinia, 2011; Tikhonov et al., 2006).

Chitosan is a commercially interesting compound because is a very abundant in nature with relatively low cost, but it also has a limited use due to its reactivity and processability (Rabea et al. 2003). This polymer is insoluble in aqueous media at neutral and basic conditions, but is soluble in aqueous diluted acids (Tikhonov et al., 2006, Hafdani & Sadeghinia, 2011). However, this solubility depends on the pKa of acids and their concentrations (Chung et al., 2005). The high molecular weight of chitosan, its high viscosity and its insolubility in aqueous solutions at neutral and basic pH (Meyer *et al.*, 2009), not

only affect its biological properties, but also constitute a limitation on certain applications in food industry. Thus, in recent years, numerous studies have been carried out to prepare modified chitosan to increase its solubility in water in order to broaden its application (Hafdani & Sadeghinia, 2011). Chitosan derivatives can be prepared by chemical derivatization under mild conditions of its reactive functional groups, an amino group as well as both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively (Silva et al., 2006, Hafdani & Sadeghinia, 2011; Xia et al. 2010). Modification of chitosan through the formation of covalent bonds with the reactive amino groups, is the most used strategy to solve these limitations, since is the more reactive functional group. Modification of chitosan through reaction with their secondary hydroxyl groups requires more steps such the protection of the functional amino groups, in order to avoid its modification.

Because of its hydrophilic nature, carbohydrates and specifically mono-and disaccharides, have been the compounds most preferred to carry out these modifications, and it has been established that substitutions of 3-20% of the amino groups leads to a drastic increase of solubility, maintaining or acquiring new biological activity, since of the remaining amino groups are not reactive (Il'ina and Varlamov, 2007). The preparation of glycosylated derivatives of chitosan, involves, among other reactions, the Maillard reaction, reductive alkylation (the Maillard reaction in the presence of sodium borohydride) and amide formation, among others. The Maillard reaction is a well-known chemical reaction of condensation between the carbonyl group of reducing sugars, aldehydes or ketones and amino groups of amino acids, proteins or nitrogenous compounds and usually requiring heat (Chang et al., 2011; Jokic et al., 2004; Kanatt et al., 2008b). Owing to the presence of a large number of amino groups in the chitosan molecule this can be involved in the Maillard

reaction. The optimal solubility and yield of chitosan derivatives depend on the reaction temperature, reaction time, the type and amount of saccharide used (Ying et al., 2011).

To date, some studies have indicated that the Maillard reaction is promising and facile for commercial manufacture of water-soluble chitosans (Chung et al., 2005; 2006; Ying et al., 2011).

By reductive alkylation (Schiff base formation and reductive N-alkylation) it is possible to obtain branched chitosan with modified functional properties or even to induce some new chemical and biological properties. To expand the range of solubility, chitosan derivatives have been prepared with mono-, di-, tri- and polysaccharides using cyanoborohydride for the reduction, which presented an excellent solubility in water (Yalpani and Hall., 1984; Yang *et al.*, 2002). Using sodium borohydride, N-alkyl derivatives of chitosan have been prepared with better fungicidal and insecticidal properties than native chitosan (Rabea et al., 2006). Cyanoborohydride has been used for applications of chitosan outside the food sector, in which the presence of trace amounts of cyanide does not represent a problem. On the other hand sodium borohydride allows the formation of derivatives in which the presence of boron in trace amounts can be admitted in food ingredients at similar levels to those present in a number of foods and not exceeding the recommended maximum level (Li and Zhang 2007).

3. Biological and Functional properties of chitosan as a food ingredient

As it was mentioned before, chitosan and its derivatives have been attracting great attention from researchers in recent years by its functions and biological activities. Among all these properties chitosan presents antitumor, cholesterol-lowering, antibacterial and antifungal effects, and other important health beneficial effects against many health problems such as cardiovascular, liver and neurodegenerative diseases. In particular for its hypocholesterolemic activity several research works have been reporting its capacity of

decreasing the levels of serum cholesterol *in vitro* (Anraku et al., 2011, Liu et al., 2008, Muzzarelli et al., 2006), *in vivo* in animals (Xia et al., 2010, Gallaher et al., 2000, Yao et al. 2008) and in humans (Maezaki et al., 1993, Gallagher et al. 2002). However it is known that these biofunctional properties depend largely on its molecular weight and acetylation degree (Lin *et al.*, 2009). This behavior along with its biocompatibility, biodegradability and absence of toxicity, has led to the chitosan being used in such diverse fields as technology, food, cosmetics, medicine, biotechnology, agriculture and the paper industry (Tharananthan and Kittur, 2003; Kim and Rajapakse 2005; Kurita 2006). In the field of food applications its antimicrobial capacity is maybe the most useful property.

Thus, in this field, different studies, developed along the last years about the antimicrobial activity of chitosan, have showed that this special polymer is able to inhibit the growth of the most common food pathogens and spoilage microorganisms. Its antibacterial activity has been reported against *Listeria monocytogenes* (Bento et al. 2011; 2009; Beverly et al., 2008; No et al., 2002), *Staphylococcus aureus* (Fernandes et al., 2008; Kanatt et al., 2008a, 2008b; Mahae et al., 2011; No et al., 2002; Rodríguez-Núñez et al., 2012; Zheng & Zhu, 2003), *Escherichia coli* (Fernandes et al., 2008; Jeon et al., 2001; Liu et al., 2006; Li et al., 2010; Mahae et al., 2011; No et al., 2002; Zheng & Zhu, 2003), *Pseudomonas* (Mahae et al., 2011; Ortega-Ortiz et al., 2010; Kanatt et al., 2008b), *Salmonella typhimurium* (Rodríguez-Núñez et al., 2012) and *Bacillus cereus* (Kanatt et al., 2008a, 2008b; Mahae et al., 2011), as well as yeasts and fungi (Tikhonov et al., 2006; Xia et al., 2010).

The antimicrobial activity of chitosan, however depends on several factors, such as its concentration, its molecular weight, matrix pH, and type of the microorganism (Georgantelis et al., 2007; Hafdani & Sadeghinia, 2011). There are different hypotheses about the mechanisms by which chitosan exert its antimicrobial activity. One of them

establishes that such activity may be due to the interaction of chitosan with membranes or cell wall components of the microorganism (Young et al., 1982; Chung et al. 2004). Usually, in the case of high molecular weight chitosan, this interaction results in the formation of a surface on the cell membrane, which prevents the entry of nutrients, causing, in consequence an inhibition of the microorganism growth. In the case of low molecular weight chitosan, the molecule could enter into the cell and cause disturbance to the physiological activities of the bacteria (Zheng & Zhu, 2003). In addition to these hypotheses, it has also been reported that chitosan can cause an increase in membrane permeability and consequently a leakage of the cell material, as well as inhibition of various enzymes (Young et al., 1982) together with a limitation of nutrients availability for bacteria (Darmadji & Izumimoto, 1994).

Another important property of chitosan and its derivatives for food applications is its antioxidant activity, reported by several authors (Kanatt et al. 2008b; Kim & Thomas, 2007; Mahae et al., 2011; Xia et al. 2010; Xie et al., 2001) and which is considered of interest for using in food preservation (Sayas-Barberá et al. 2011) in particular in products with a high lipid content. The inhibitory effect of lipid oxidation by the antioxidant effect of chitosan can be explained by its ability to chelate iron ions (Shahidi et al. 1999). Xie et al. (2001) studied the antioxidant activities of water-soluble chitosan derivatives and reported that the scavenging mechanism may be related by the fact that the free radicals can react with active hydrogen atoms in chitosan to form a most stable macromolecule radical.

As every property of chitosan, its antioxidant activity depends on its MW and DD. Chien et al. (2007) assessed the effect of molecular weight of different chitosans on their antioxidant activities and they found that the low molecular weight chitosan (LMWC) exhibited the highest radical scavenging activity, followed by medium molecular weight chitosan (MMWC) and high molecular weight chitosan (HMWC). This behaviour may be

explained by the formation of intramolecular hydrogen bonds into the chitosan molecule (Feng et al., 2007). Thus, into the HMWC, a high number of intramolecular hydrogen bonds would exist and chitosan would have lower mobility than the LMWC, which would increase the possibility of inter and intramolecular bonding among the HMWC molecules. Thus, the chance of exposure of their amine groups (-and OH) might be restricted, which would account for less radical-scavenging activity (Kim & Thomas, 2007).

Regarding to the water-soluble chitosan derivatives different research studies have indicated their potential application as promising commercial substitutes for acid-soluble chitosan (Chung et al., 2006) not only due to the improved technological properties but also due improved biological properties. Thus, Kanatt et al. (2008) reported that the chitosan-glucose complex has significantly higher antibacterial and antioxidant activity than chitosan alone. Similar results were reported by Mahae et al. (2011), which indicated the chitosan-sugar complexes as an alternative natural product for using as replacement of synthetic food additives.

Additionally to its antimicrobial and antioxidant capacity, chitosan possess other interesting properties for its application on foods, such as lipid and water binding capacity (Knorr, 1983; Nauss & Nagyvary, 1983; Sayas-Barberá et al. 2011) and emulsification (Lee, 1996), which improves functional and technological features. In this context, some studies can be found on the fat reduction in meat products by the incorporation of chitosan (Amaral et al. 2015; Kim & Thomas, 2007, Lin & Chao, 2001).

4. Application of chitosan and its derivatives in meat and meat products

Meat is the muscle tissue of slaughter animals composed of water, proteins, lipids, minerals and a small proportion of carbohydrates (Shah et al., 2014). Thus, due to their rich nutritional composition and high level of water the meat and meat products are susceptible

to quality deterioration (Devatkal et al. 2012). Together with microbial spoilage, chemical deterioration, especially lipid oxidation, is the main factor limiting the shelf-life of muscle foods (Kanatt et al. 2013). Lipid oxidation is a reaction responsible for impairing nutritional properties of foods since it involves the loss of essential fatty acids and vitamins and the generation of toxic compounds as the malondialdehyde (MDA), as well as, affects sensory traits of meat product, causing flavor, color and texture deterioration (Estévez et al., 2005). At the same time, the microbial growth may cause both spoilage and food borne diseases (Georgantelis et al., 2007). Thus, nitrate and nitrite are curing agents widely used in meat products, since they can have a significant contribution towards the extension of shelf life by reducing lipid oxidation, limit microbial growth and at same time promote final meat colour. (Honikel, 2008; Georgantelis et al., 2007). However, the utilization of nitrite has been limited because it results in the formation of N-nitrosamines, a group of compounds that are well known for their carcinogenic and mutagenic activities (Wang et al., 2015). These risks have created a need for developing alternative preservation systems for meat and meat products (Sagoo et al., 2002, Sayas-Barberá et al. 2011).

Chitosan has showed to possess several biological and functional properties of great interest for the Food Industry. This fact, together with the increased demand (by the consumers) of using natural preservatives in food, have caught the attention from the industry and from the research community to study the feasibility of the incorporation of chitosan (Table 1) or their application as coating or film (Table 2) either individually, or in combination with other natural agents in different kinds of meat and meat products. Thus, although it is necessary to conduct further studies, it is already possible to evaluate the positive impact of this polymer in biological and technological properties of the treated products.

Meat: The effect of chitosan in meat preservation including microbiological, chemical, sensory and color qualities have been documented. Darmadji & Izumimoto (1994) reported that chitosan (0.5 – 1.0%) in meat, during incubation at 30°C for 48h or storage at 4°C for 10 days, inhibited the growth of spoilage bacteria (*Staphylococci*, *Coliform*, Gram-negative bacteria, *Micrococci* and *Pseudomonas*), reduced lipid oxidation and putrefaction, and resulted in better sensory attributes, as well as also had a good effect on the development of the red color of meat during storage. The study of Juneja et al. (2006) to investigated the inhibition of *Clostridium perfringens* spore germination and outgrowth by the biopolymer chitosan during abusive chilling of cooked ground beef (25% fat) and turkey (7% fat) reported that addition of chitosan (3%) reduced by 4 to 5 log₁₀ CFU/g of *C. perfringens* spore germination and outgrowth during exponential cooling of the cooked beef or turkey. Thus, the results suggest that incorporation of 3% chitosan into ground beef or turkey may benefit microbial food safety and the consumer, due to reduce the potential risk of *C. perfringens* spore germination and outgrowth during abusive cooling from 54.4 to 7.2 °C in 12, 15, or 18 h.

Furthermore, the effect of chitosan has been evaluated in combination with other natural agents. Kanatt et al. (2008a) demonstrated the efficacy of chitosan together to a mint mixture as a potent antibacterial and antioxidant agent that could be used for the preservation and shelf life extension of meat and meat products. Petrou et al. (2012) evaluated the chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. Their results demonstrated that the combined use of chitosan and oregano essential oil could inhibit the growth of microbial spoilage flora, retard lipid oxidation, maintain lightness and improve the sensory quality of fresh chicken meat. Similar behavior was reported by Vasilatos & Savvaidis (2013), who evaluated the effect of chitosan and rosemary essential oil on the quality characteristics of fresh turkey meat under vacuum

packaging conditions. They reported that combination of chitosan and rosemary essential oil could inhibit growth of microbial spoilage flora and improve the sensory quality of fresh turkey meat stored under refrigeration (2 °C).

Ready-to-eat (RTE) meat products: Applications of chitosan for inhibiting *Listeria monocytogenes* have been documented in various meat products Ready-to-eat (RTE). These products are generally exposed to a thermal treatment with the main objective of eliminating pathogens. However, they may be again contaminated in a next step, as a slicing process (Moradi et al. 2011). Bento et al. (2011) evaluated the efficacy of chitosan from *Mucor rouxii* UCP 064 in inhibiting *Listeria monocytogenes* and the influence of the chitosan addition on sensory aspects in bovine meat pâté at 4 °C. Their results suggested that chitosan from fungi could be considered a possible alternative compound to control *L. monocytogenes* in meat products. It would also be acceptable by consumers, although some negative influence on flavor and taste could be noticed. Beverly et al. (2008) demonstrated that edible chitosan films dissolved in acetic acid or lactic acid could be useful as coatings to control *L. monocytogenes* on the surface of RTE roast beef. Moradi et al. (2011) evaluated the effectiveness of *Zataria multiflora* Boiss essential oil (ZEO) and grape seed extract (GSE) impregnated chitosan film on ready-to-eat mortadella-type sausages during refrigerated storage. They reported that all films exhibited antibacterial activity against *L. monocytogenes* on agar culture media and indicated that chitosan films could be developed by incorporating GSE and ZEO for extending the shelf life of mortadella sausage. Besides the mentioned bacterial, the studies of Shekarforoush et al. (2015) indicated that chitosan in combination with oregano essential oil also can reduce the number of spoilage and safety indicator bacteria and *E. coli* O157:H7 4b in RTE barbecue chicken meat.

Salame: The shelf life of salami is generally limited due to the microbiological growth and lipid oxidation. Kanatt et al. (2008a) evaluated the application of a mixture of chitosan and mint as a preservative of pork cocktail salami. Considering the total bacterial counts, they reported that control salamis spoiled in less than two weeks, while salamis treated with chitosan and mint mixture displayed a shelf life of three weeks at chilled temperature (3°C) and were relatively resistant to lipid oxidation. The efficiency of chitosan to also control lipid oxidation in meat and meat products has been previously reported by several authors (Darmadji & Izumimoto, 1994; Shahidi et al., 1999; Soultos et al. 2008; Georgantelis et al. 2007). This inhibitory effect can be explained by the ability of chitosan to chelate iron ions (Shahidi et al. 1999).

Burger: The use of chitosan in the manufacture of burgers has a great potential for the enhancement of stability and preservation, which may allow a reduction of the use of synthetic preservatives in meat, as well as improving the cooking properties of burgers, preserving their quality and extending their shelf life (Sayas-Barberá et al., 2011).

Sausage: Many studies have investigated the effect of chitosan as a natural ingredient that can be used effectively to extend the shelf-life of sausages. Sagoo et al. (2002) reported that chitosan increased the shelf-life of chilled skinless sausages from 7 to 15 days. Their results indicated that the addition of 0.3 and 0.6% chitosan reduced total viable counts, yeasts and moulds, and lactic acid bacteria by up to 3 log cfu g⁻¹ for 18 days at 4°C compared with the untreated control. Similarly, Bostan & Mahan (2011), reported that application of chitosan (0.25%, 0.5% and 1.0%) on the sausage surface by dipping improved the microbiological quality and did not cause undesirable alteration in sensory properties of sausages. Soultos et al. (2008) reported that both the antimicrobial and antioxidant effects of chitosan were more pronounced when using it at a concentration of 1% rather than 0.5%. Thus extend the shelf-life of fresh pork sausages stored under chill conditions.

With respect the concern for the reduction of synthetic additives and the impact on quality, García et al. (2011) to evaluate partial substitution of nitrite by chitosan reported that chitosan increased significantly the shelf life of pork sausages without affecting sensory attributes. Amaral et al. (2015) indicated that chitosan possesses an interesting potential to be included in fresh pork sausages, since it causes an increase in the stability and shelf-life of the product, considering the reduction of microbial growth and lipid oxidation, assuring at same time a best red color and a more stable emulsion, by the ability to bind water and fat, and without negatively affecting the sensory properties. Georgantelis et al. (2007), demonstrate the effectiveness of chitosan, added individually or in combination with rosemary extract on microbial growth inhibition, retarding lipid oxidation and extending shelf life of fresh pork sausages of the traditional Greek-type. They reported that this combination could improve preservation of these products without the use of nitrites or other additives, promoting consumer health and improving consequently commercial value final products.

Additionally, some research can be found on fat reduction in meat products by the incorporation of chitosan without causing adverse effects in sensory characteristics and textural properties (Amaral et al., 2015; Lin & Chao, 2001). It is well known the amount and the type of fat consumed is associated with the risk of causing coronary heart disease (Lin & Chao, 2001). Therefore, reformulation of meat products based on processing strategies is an important trend to develop products that can promote a better consumer health (Jiménez-Colmenero et al., 2010). Amaral et al. (2015) reported that reduction of fat content to levels of 5% can be positively achieved with the incorporation of chitosan in fresh pork sausage.

Finally, research has shown the potential application of chitosan in the obtaining of healthier meat products. Amaral et al. (2015) indicated that incorporation of 2% chitosan in pork sausages can assure the ingestion of 3 g of chitosan per day (3 sausages) and to

accomplish, thus, the EFSA claims about cholesterol reduction. Their work showed as this incorporation is technologically feasible, allowing the obtaining of a product with improved properties. However, Grasso et al. (2014) reported that it is still uncertain if the meat industry will be able to successfully enter the functional food market, with many challenges lie ahead, such as: meat and meat products are not a traditional matrix for the delivery of functional ingredients and they pose significant technological challenges; the meat industry will need to provide exceptional quality raw materials and engage in innovative marketing strategies; a high level of scientific evidence to support the health enhancing properties is required.

Coating or Film: In addition to the incorporation of chitosan into food matrices, a great number of studies have been developed with the objective of using chitosan as a coating and also, as a part of edible films, frequently used for food protection. The inherent antibacterial/antifungal properties and film forming ability of chitosan make it ideal for its use as biodegradable antimicrobial packaging material that can be used to improve the storability of perishable foods (Dutta et al., 2009, No et al., 2007; Siripatrawan & Noipha, 2012). Thus, this polymer has received a significant attention as film-forming agent for food preservation to the researchers due to its biodegradability, biocompatibility, cytotoxicity, and antimicrobial activity (Dutta et al. 2009).

Food packaging continues to be one of the most important and innovative areas in development of new processes and products, because, besides requiring protection, specific physical-chemical properties and attractive esthetical look, advanced food packaging materials function as a preservation system (Zemljic et al. 2013). Therefore, studies indicate that the future is prominent and, in a short period of time, chitosan will be a usual ingredient used in the food industry.

Siripatrawan & Noipha (2012) concluded that the developed active film could be used as active packaging to maintain qualities and extend shelf life of the refrigerated pork

sausages. Kanatt et al. (2013) reported that the chitosan coating significantly reduced the growth of *B. cereus*, *E. coli*, *P. fluorescens* and *S. aureus* in ready-to-cook meat products. Thus, they indicated that chitosan coating could be employed as an active packaging in meat industry to control postprocessing microbial contaminants, and, therefore, to improve the safety of meat products. As well as, Qin et al (2013) who investigate the effect of a chitosan (CH) film containing also tea polyphenols (TP) on quality and shelf life of pork meat patties stored at 4 ± 1 °C for 12 days. This study demonstrated a microbiological shelf-life extension of 6 days, a decrease in the thiobarbituric acid-reactive substances (TBARS) values and in the metmyoglobi (MetMb) content and a maintenance in the sensory qualities throughout the storage for treated samples when compared to the control group. In view to these results, the authors concluded that CH-TP composite film could be a promising material as a packaging film for extending the shelf life of pork meat patties.

The effectiveness of the antimicrobial and antioxidant activity of chitosan coatings was also observed individually or in combination with other natural agents. Thus, Pranoto & Rakshit (2008) reported that using a chitosan coating on meatballs significantly reduced the microbial growth during the storage and highlighted that the use of garlic oil, when incorporated into chitosan coating, provided it an additional antioxidant activity. Lekjing (2016) investigate the effect of chitosan (2%) coatings, with (1.5%) and without clove oil, on the quality and shelf life of cooked pork sausages stored under refrigeration at a temperature of 4 ± 2 °C. Their results demonstrated that the combination of chitosan and clove oil got inhibit the microbial growth (total viable counts and psychrotrophic bacteria), retard the lipid oxidation, and extend the shelf life of cooked pork sausages storage under refrigeration. Gilani et al. (2015), in another recent and interesting study, reported that a chitosan (CH) coating enriched with *Zataria multiflora* essential oil (ZEO) significantly decreased total viable counts, *Pseudomonas* spp., lactic acid bacteria, Enterobacteriaceae,

Psychrotrophic bacteria and yeasts-molds as compared with the control samples during the storage period. Peroxide values, thiobarbituric acid reactive substance values and protein oxidation were also significantly lower. Therefore, chitosan-based edible coatings developed can be used to increase the shelf life and to improve the microbial safety parameters of meat and meat products (Baranenko et al, 2013).

6. Conclusion

This review summarizes the different studies carried out, to date, on the application of chitosan as a natural ingredient in food matrices, especially in meat products. Despite the main advantage that chitosan may provide to foods – their antimicrobial action, chitosan has showed to possess many other interesting properties such as antioxidant and hypocholesterolemic effect. Although, in the context of a healthy diet, it is necessary to conduct further studies, the addition of chitosan it is well documented and proved. It can act positively on the quality and shelf-life of food and specifically, in meat and meat products, without offering risk for consumer health and without adversely affecting sensory characteristics. Therefore, the use of chitosan could result in functional healthy products technologically feasible.

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Table 1- Summary of studies testing the impact of chitosan/chitosan derivatives in meat and meat products.

Meat/meat product	Chitosan	Stored conditions	Technological results	Biological results	References
Cured chicken meat	Chitosan solution (2%) individually and chitosan solution (2%) + oregano	Stored at 3, 8 and 20 °C		Chitosan: -No inhibitory effect on spoilage-inducing bacteria and <i>E. coli</i> -Effective against <i>L. monocytogenes</i> Chitosan+oregano -Reduce the number of spoilage and safety indicators and also the two food-borne pathogens	Shekarforoush et al. (2015).
Low fat fresh pork sausage	Chitosan (2%)	Stored for 15 days at 4°C	Reduction of lipid oxidation, best red color, ability to bind water and fat and not affect negatively the sensory properties. The results showed also that, the reduction of fat content to levels of 5% was positively achieved with the incorporation of chitosan	Reduction of microbial growth	Amaral et al. (2015).
Pork burgers	Chitosan of high and low molecular weights (0%, 0.25%, 0.5% and 1%)	Stored for 8 days at 4 °C	Increased the shelf life of burgers, extends the red color, improves all cooking characteristics	Reduced total viable counts and antioxidant activity	Sayas-Barberá et al. (2011).
Ham Visking fresh pork sausages	Partial nitrite replacement by chitosan	Stored for 35 days at 4 °C	Not affect sensory attributes and significant increase of shelf life.		García et al. (2011).
Vacuum-packaged fresh pork sausages	Chitosan (1%)	Stored for 26 days at 4 °C	Not affect texture and sensory attributes.	Microbial growth was not inhibited	García et al. (2010).

Greek style fresh pork sausages	Chitosan (0.5% and 1%) added individually or in combination with nitrites (150 ppm)	Stored for 28 days at 4 °C	Although the quality of the sausages with added chitosan was acceptable, the addition of nitrites was deemed necessary to obtain sausages with improved sensory attributes.	Both the antimicrobial and antioxidant effects of chitosan were more pronounced when using it at a concentration of 1%.	Soultos et al. (2008).
Fresh pork sausages	Chitosan added individually or in combination with rosemary and α -tocopherol extract	Stored for 20 days at 4 °C	Retarding of lipid oxidation and shelf life extension.	Microbial growth inhibition	Georgantelis et al. (2007).
Chilled pork sausages	Chitosan (0.6%) and carnocin in combination with low concentrations of sulphite	Stored for 24 days at 4 °C	The batch containing chitosan and sulphite deteriorated less rapidly and was judged to be more acceptable for a longer period than all the other batches.		Roller et al. (2002).
Reduced-fat Chinese-style sausage	Chitosan 0.1% with various molecular weights (CHL, CHM, CHH)	Stored for 9 days at 4 °C	Chitosan allowed reduce-fat (~22%) resulted in better or similar quality regarding physicochemical, microbial and sensory characteristics with no adverse effect on textural properties. The authors reported a possible application of so-called “healthy” food additive chitosan to Chinese-style sausage.		Lin & Chao (2001).
Cooked pork sausage	Water-soluble chitosan oligomer (0.2%)	Stored for 3 weeks at 4 °C	No adverse effect on quality aspects was showed		Jo et al. (2001).
Bovine pâté	5 mg chitosan/g pâté	Stored for 6 days at 4 °C	Sensory evaluation suggested that addition of chitosan in pâté would be acceptable to consumers, although some negative influence on flavor and taste was found	Decreased the counts of <i>L. monocytogenes</i> from approximately 7 to 3 log cfu/g	Bento et al. (2011).
Meat minced	Chitosan (0%, 0.2%, 0.5% and 1%)	One batch of meat was incubator at 30°C for 48h and the other was placed at 4°C for 10 days	Reduced lipid oxidation, resulted in better sensory attributes and development of the red color	Inhibited the growth of spoilage bacteria	Darmadji & Izumimoto (1994).

Table 2- Summary of studies testing the impact of chitosan application as coating or edible film in meat and meat products.

Meat/meat product	Chitosan treatments	Stored conditions	Technological results	Biological results	References
Ground meat	Film prepared with 1% (w/0.6v) chitosan, (w/wCHT) n-cellulose and 30% (v/wCHT) glycerol	Stored for 6 days at 4 or 10°C		Decreased lactic acid bacteria population up to 3.1 logarithmic cycles	Dehnad et al. (2014).
Ready-to-eat deli turkey meat	Chitosan coating (2 and 5%) in an acetic, lactic and levulinic acids (2%).	Stored at -20°C and prior to experiments were thawed overnight at 4°C		Reduction of <i>Listeria innocua</i>	Guo et al. (2014).
Ready-to-eat mortadella-type sausages	Chitosan films containing <i>Zataria multiflora</i> Boiss essential oil (ZEO) (5 and 10 g kg ⁻¹) and grape seed extract (GSE) (10 g kg ⁻¹).	Stored for 21 days at 4°C	-Retard lipid oxidation; - Extends the shelf-life	Inhibits the microbial growth: - <i>L. monocytogenes</i> - Lactic acid bacteria - Aerobic mesophiles bacteria	Moradi et al. (2011).
Chicken balls, chicken seekh kababs and mutton seekh kababs	Chitosan coating (2 g/100 ml) in acetic acid (1%)	Stored for 14 days at 3°C	Retarded lipid oxidation, as well as, longer shelf life and no significant organoleptic changes	-Chitosan coating eliminated fecal coliforms and lowered counts of <i>Staphylococcus spp.</i> -Inhibit growth of microbial of <i>Bacillus cereus</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> and <i>Pseudomonas fluorescens</i> .	Kanatt et al. (2013).
Dry fermented sausage	Chitosan-caraway coating (4 kg m ⁻³) in acetic acid (1%)	Stored for five months	Lower intensity of lipid oxidative and better sensory properties		Krkic et al. (2013).

Chicken breast meat	Chitosan dipping or oregano oil treatments (1.5% w/v) in glacial acetic acid (1%) combined with oregano oil (0.25% v/w)	Modified atmosphere packaged and stored for 24 days at 4 °C	Retard lipid oxidation, maintain lightness and improve the sensory quality	Chitosan + oregano oil: -Inhibit growth of microbial of mesophilic Total Plate Counts (TPC), lactic acid bacteria (LAB), Brochothrix thermosphacta, Enterobacteriaceae, Pseudomonas spp. and yeasts-moulds	Petrou et al. (2012).
Pork meat hamburgers	Edible films of chitosan (1%) in an acetic or lactic acid (1%) combined with sunflower oil (1%) edible films	Stored for 8 days at 4 °C		Chitosan films led to a reduction of total viable and coliform counts of samples during storage	Vargas et al. (2011).
Sausage	Chitosan coating (0.25, 0.5 and 1%) in acetic acid	Stored for 60 days at 4 °C	Extends the shelf-life	Inhibits the microbial growth: -0.25% for aerobic mesophilic bacteria and psychrotrophic bacteria; -0.5 and 1% for lactic acid bacteria and yeast-mold, respectively.	Bostan & Isin Mahan (2011).
Sliced fresh red meats	Chitosan-incorporated LDPE film (0, 1, 4 and 8 %)	Stored for 10 days at 3 °C	Significant extension of red color shelf life were observed in refrigerated	When chitosan incorporated films were applied on fresh red meat, microorganisms on the meat surface were not inhibited (total aerobic cell populations)	Park et al. (2010).
Meatball	Chitosan coatings (1%) were incorporated with garlic oil (0.2%), potassium sorbate (0.1%) and nisin (51.000 IU)	Stored for 60 days at 4 °C	Reduction of rancidity level and lower moisture loss	All chitosan coatings suppressed total microbial growth	Pranoto & Rakshit (2008).
Chilled pork products	Sausages were dipped in chitosan solutions (1%) in deionized water	Standard sausages (1, 2 or 3% for 0, 15 or 30 min) and skinless sausages (0.5 or 1% for 0, 1, or 30 min). After were drained, packed and stored at 7, 15 and 25 °C and analyzed for 18 days	Chitosan treatment increased the shelf-life of chilled skinless sausages from 7 to 15days	Chitosan was an effective inhibitor of microbial growth: -1% reduced the native microflora (total viable counts, yeasts and moulds, and lactic acid bacteria) by approximately 1-3logcfug ⁻¹ for 18 days at 7°C.	Sagoo et al. (2002).

3. MATERIAL E MÉTODOS

3.1. Ingredientes da salsicha e quitosana

A carne crua (suína e caprina) e a gordura, os ingredientes (sal, alho fresco, pimenta branca em pó e orégano) e a tripa artificial foram obtidos no mercado local na cidade do Porto (Portugal) para execução dos estudos realizados na Escola Superior de Biotecnologia da Universidade Católica Portuguesa - ESB/UCP (estudos 1 e 2), e em mercado público da cidade de Petrolina (Brasil) para execução do estudo desenvolvido na Universidade Federal da Paraíba - UFPB (estudo 3). A Quitosana de Baixo Peso Molecular (LMWC, Sigma-Aldrich, Steinheim, Alemanha) utilizada apresentava Peso Molecular (PM) de 123 kDa e 90% de grau de desacetilação (MONTENEGRO, 2013). O derivado da quitosana (LMWC-Glc) foi produzido em laboratório como descrita no item 2.1.1.

3.1.1. Síntese do derivado

O derivado da quitosana foi sintetizado segundo a metodologia descrita em Montenegro (2013) seguindo as condições ótimas de síntese. Para isso o LMWC foi dissolvido em solução de ácido acético a 1% (v/v) para ter uma concentração final de 2% (m/v). A glucose (Glc) a 2% (m/v) foi adicionada na solução de quitosana. A reação foi mantida num banho de água a 60 °C por 32 h sob agitação (100rpm) (Figura 1A) para que ocorresse a reação de Maillard (Figura 1B), a qual foi finalizada em banho de gelo por 10 minutos. Em seguida, o produto da reação foi dialisado (membrana de peso molecular de 3500 Da, Thermo Scientific Inc., USA) em água destilada por 2 dias (Figura 1C), depois ajustou-se a pH 7 com NaOH a 1% (m/v), sendo em seguida congelado e por fim liofilizado (Figura 1D). O derivado da quitosana foi mantido sob refrigeração a 4 °C até ser utilizado na elaboração das salsichas. A caracterização correspondente do derivado pelas técnicas correspondentes indicou um PM de 210.37 KDa e um grau de substituição por parte da Glc, de 65%, com 25%, a percentagem de grupos amino livres.

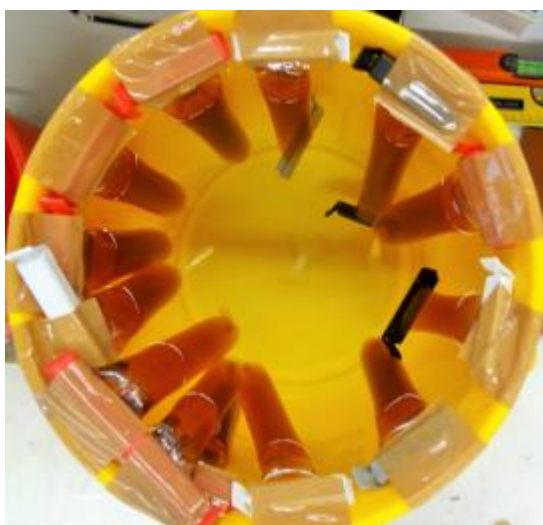
A.



B.



C.



D.



Figura 1. Solução de LMWC com Glc antes da reação (A) e depois da reação (B). Diálise em água destilada (C) e produto final, LMWC-Glc (D) obtido após liofilização.

3.2. Delineamento Experimental

Três estudos (Figura 2) foram realizados utilizando carne suína e caprina, bem como diferentes teores de gordura suína (Tabela 1). A concentração da quitosana foi selecionada de modo a atender os requisitos da Autoridade Europeia para a Segurança dos Alimentos (EFSA), a qual indica que 3 g de quitosana por dia em uma ou mais porções pode proporcionar o efeito hipocolesterolêmico. Assim, a quitosana de baixo peso molecular (LMWC) e a quitosana modificada com glucose (LMWC-Glc) foram adicionados a 2% (m / m) nos três estudos para assegurar o consumo de 3 g de quitosano em uma porção de salsichas de 150 g, correspondendo a 1 g de quitosana em uma salsicha de cerca de 50 g cada.

De acordo com o regulamento técnico de identidade e qualidade de salsicha (BRASIL, 2000) o teor máximo de gordura permitido é 30%. Portanto, justifica-se a escolha dos teores de gordura avaliados nos estudos 1 e 2 (20%, 12,5% e 5% m/m) objetivando a redução de cerca de 34%, 58% e 83% do conteúdo de gordura, respectivamente. Já no terceiro estudo (10% m/m de gordura) a redução estudada foi de cerca de 66% do teor de gordura, assim buscando desenvolver um produto mais saudável.

1º Estudo: Na elaboração da salsicha foi utilizada carne suína com produção de 3 lotes distintos, nos quais foram estudadas três concentrações de gordura com adição de 2% de LMWC, tais como: 5% (m / m) (Formulação 1B), 12,5% (m / m) (Formulação 2B) e 20% (m / m) (Formulação 3B). Para cada nível de gordura foi produzido outro lote sem quitosana utilizada como controle para cada formulação (1A, 2A e 3A). As amostras foram armazenadas sob refrigeração a $4 \pm 1^{\circ}\text{C}$ por 15 dias, sendo submetidas a análises microbiológicas, físico-químicas e sensoriais a cada 5 dias.

2º Estudo: Foi utilizada carne caprina removida dos cortes da costela, pescoço e barriga, de animais machos, sem raça definida, abatidos entre 8 e 10 meses de idade para a produção de 3 lotes distintos, nos quais foram estudadas três concentrações de gordura com adição de 2% de LMWC, tais como: 5% (m / m) (Formulação F5C), 12,5% (m / m) (Formulação F12,5C) e 20% (m / m) (Formulação F20C). Para cada nível de gordura foi produzido outro lote sem quitosana utilizada como controle para cada formulação (F5, F12,5 e F20). As amostras

foram armazenadas sob refrigeração a $4 \pm 1^\circ\text{C}$ por 15 dias, sendo submetidas a análises microbiológicas e físico-químicas a cada 5 dias.

3° Estudo: Na elaboração da salsicha foi utilizada carne caprina de animais machos sem raça definida (SRD), abatidos entre 8 e 12 meses de idade. Todos os tratamentos foram elaborados com apenas uma concentração de gordura 10% (m / m), sendo produzidos 3 lotes com a variação do tipo de quitosana, nomeadamente: LMWC, LMWC-Glc e uma formulação controle sem quitosana e derivado da quitosana. As amostras foram armazenadas sob refrigeração a $4 \pm 1^\circ\text{C}$ por 21 dias, sendo submetidas a análises microbiológicas, físico-químicas e sensoriais a cada 7 dias. O período de armazenamento foi prolongado, diferindo dos estudos 1 e 2, para observação da influência do tempo sob a ação dos polímeros adicionados na salsicha.

Tabela 1 – Formulações das salsichas realizadas em cada estudo.

Matéria-Prima / Ingredientes (%)	1° Estudo Salsicha Suína	2° Estudo Salsicha Caprina	3° Estudo Salsicha Caprina
Carne picada	77	77	75
Água gelada	10	10	10
	5	5	
Gordura suína	12,5	12,5	10
	20	20	
Quitosana (LMWC)	2	2	2
Derivado (LMWC-Glc)	-	-	2
Sal	1,5	1,5	1,5
Alho	1,3	1,3	0,1
Cebola	1,3	1,3	0,2
Pimenta	0,2	0,2	0,1
Orégano	0,1	0,1	0,05
Amido (fécula de mandioca)	-	-	2
Proteína Isolada de soja	-	-	2

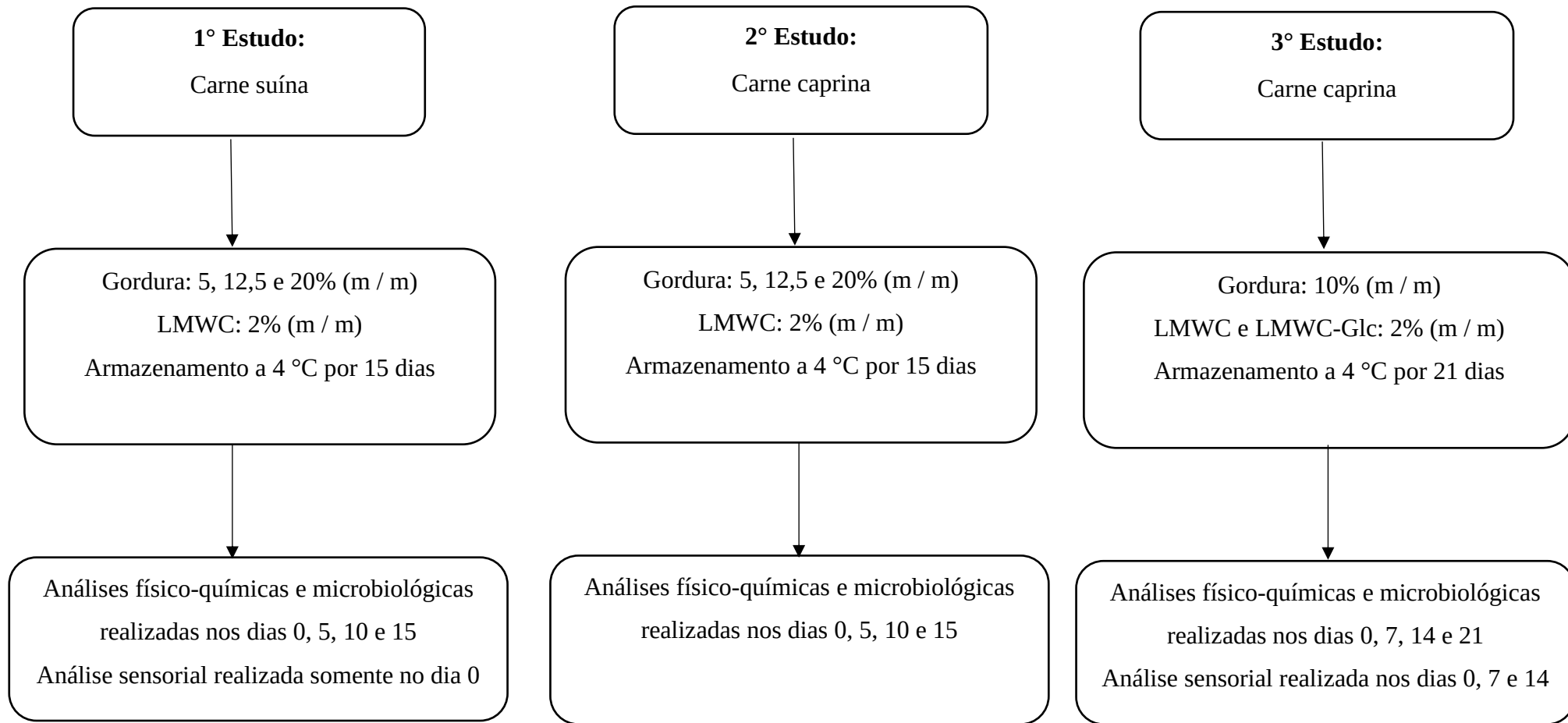


Figura 2. Delineamento experimental dos estudos realizados com salsichas suínas e caprinas adicionadas de quitosana ou quitosana modificada ao longo do armazenamento refrigerado a 4 ± 1 °C.

3.3. Processamento da Salsicha

1° e 2° Estudos:

A salsicha fresca foi preparada de acordo com a formulação apresentada na Tabela 1. A quitosana (LMWC) foi adicionada a 2% (m / m), a fim de que se atendesse as exigências da alegação de saúde da EFSA (3 g / dia). Para a preparação das salsichas, a carne foi misturada com a gordura de porco, em seguida, foram adicionados os demais ingredientes e, finalmente, a quitosana. Após completa homogeneização dos ingredientes, e um período de espera de 3 minutos, a mistura cárnea foi incorporada em um invólucro artificial, obtendo-se salsichas frescas de 3 cm de diâmetro e 50 g por unidade. Por fim, as salsichas foram embaladas sem vácuo em sacos plásticos e armazenadas sob refrigeração a 4 ° C por 15 dias. Um lote de 1000 g de salsicha fresca de cada formulação foi preparado e dividido em duas réplicas para cada lote produzido, que foram analisados posteriormente em duplicata.

3° Estudo:

A Tabela 1 apresenta a formulação da salsicha caprina fresca. A quitosana (LMWC) e o derivado da quistosa (LMWC-Glc) foram adicionados a 2% (m / m). Uma amostra sem LMWC e sem LMWC-Glc foi utilizada como controle. Para o preparo das salsichas, a carne caprina picada foi misturada com o sal, parte da gordura e da água em um cutter (JAMAR, modelo K-10, São Paulo, Brasil), em seguida, o restante da água e da gordura foram homogeneizados juntamente com os demais ingredientes (alho e cebola em pó, pimenta preta, amido, orégano, proteína isolada de soja e quitosana ou derivado). No caso do derivado, este foi umedecido em parte da água para então ser adicionado a formulação. Após completa homogeneização dos ingredientes (Figura 3A), cerca de 3 minutos, a mistura cárnea foi incorporada em um invólucro artificial, utilizando-se uma embutidora manual tipo canhão (SIEMSEN LTDA, modelo CFMN ES-08, Brusque - SC, Brasil) com capacidade para 3 Kg (Figura 3B), assim obtendo salsichas frescas de 3 cm de diâmetro e 50 g por unidade (Figura 3C). Por fim, as salsichas foram embaladas sem vácuo em sacos plásticos e armazenadas sob refrigeração a 4 ° C por 21 dias (Figura 3D). Um lote de 1000 g de salsicha fresca de cada formulação foi preparado e dividido em duas réplicas para cada lote produzido, que foram analisados posteriormente em triplicata.

A.



B.



C.



D.



Figura 3. Sequência de etapas do processo de elaboração de salsicha fresca de carne caprina. Massa cárnea emulsionada (A). Envaze da massa cárnea em tripa artificial (B). Salsicha fresca (C). Armazenamento sob refrigeração a 4 ± 1 °C para o estudo de vida de prateleira (D).

3.4. Métodos

As salsichas elaboradas nos três experimentos foram armazenadas sob refrigeração comercial (4 ± 1 °C) e analisadas quanto à sua qualidade microbiológica, aspectos físico-químicos e sensoriais.

3.4.1. Análises Microbiológicas

Com o objetivo de avaliar a qualidade microbiológica das salsichas, contagens de bactérias aeróbias mesófilas e psicrófilas, bem como *Enterobacteriaceae* e bolores e leveduras foram analisadas durante o armazenamento a 4 ± 1 °C. Deste modo, 8 g de amostra em 80 mL de água peptonada estéril foram colocados em sacos de plástico e homogeneizadas durante 2 minutos utilizando um Stomacher (Lab Blender 400, Londres, Reino Unido). O homogeneizado foi diluído em série com água peptonada estéril e as contagens de células viáveis foram avaliadas pelo método de gota (20 µL de cada diluição), tal como descrito por Miles, Misra e Irwin (1938), exceto para as *Enterobacteriaceae*, onde se utilizou a técnica de plaqueamento em profundidade (1 mL de cada diluição). Foram utilizados condições e meios de incubação específicos para cada microrganismo. Assim, o plate count agar (PCA, Biokar diagnostics), foi utilizado para bactérias mesófilas e psicrófilas, e as placas foram incubadas a 30 °C durante 48 h e 7 °C durante 7 dias, respectivamente. Bolores e leveduras foram cultivadas em Potato Dextrose Agar (PDA, Biokar diagnostics) sendo as placas incubadas a 25 °C durante 5 dias. *Enterobacteriaceae* foram cultivadas em Violet Red Bile Glucose Agar (VRBGA, Lab) e as placas foram incubadas a 37 °C durante 24 h. Após a incubação, foi contada o número de colônias expresso em unidades formadoras de colônias (UFC / mL).

3.4.2. Análises físico-químicas

3.4.2.1. Umidade, proteína, gordura, cinzas e pH

Umidade, cinzas, proteínas e o teor de gordura das amostras foram determinadas nas salsichas cruas, durante o armazenamento a 4 ± 1 °C segundo procedimentos nº 24,003, 24,009, 24,027 e 24,005, respectivamente (AOAC, 1990). A umidade (g de água / 100 g de

amostra) foi determinada por secagem das amostras a 105 °C até peso constante. O teor de cinzas (g cinzas / 100 g de amostra) foi calculado depois da incineração das amostras em uma mufla a 550 ° C e pesado. O teor de proteína (expressos em g de proteína / 100 g de amostra) foi analisada pelo método de Kjeldahl. O teor de gordura (gordura g / 100 g de amostra) foi calculado pela perda de peso após a extração a quente com hexano num aparelho de Soxhlet. No terceiro experimento o teor de gordura foi determinado pela extração a frio com clorofórmio/metanol (2:1) pelo método de Folch, Less e Stanley (1957).

Os valores de pH das amostras foram analisados pelo método 943,02 (AOAC, 2000) utilizando um elétrico de vidro de pH ligado a um potenciômetro microph 2001 Crison (Barcelona, Espanha).

3.4.2.2. Oxidação lipídica

A oxidação de lipídica foi avaliada através da medição das substâncias reativas ao ácido tiobarbitúrico (TBARS) pelo método adaptado de Rosmini et al. (1996). Assim, 2 g de amostra foram homogeneizados em vortex em 10 mL de 10% (v / v) de ácido tricloroacético (TCA bioquímica / Applichem) e 5 ml de 0,02 M de ácido 2-tiobarbitúrico (TBA, Merck). Em seguida, foi centrifugada a 5000 rpm durante 20 min numa centrífuga Universal 320R (Zentrifugen Hettich). O sobrenadante foi recolhido e filtrado, aquecido em água em ebulição (100 °C) durante 35 minutos e arrefeceu-se em água gelada durante 10 min. Por fim, a absorbância a 532 nm foi medida num espectrofotómetro de UV mini-1240 (Shimadzu, Tóquio, Japão). O 1,1,3,3 Tetraethoxypropane (Sigma Aldrich) foi usado como padrão no intervalo de 1×10^{-6} - 14×10^{-6} mol / L. A concentração de TBARS foi expressa em mg de malonaldeído por Kg de amostra. Duas salsichas de cada tratamento foram analisadas em duplicata.

3.4.2.3. Análise de cor

A cor de cada salsicha foi determinada de acordo com a metodologia descrita por Abularach, Rocha e Felício (1998), utilizando um colorímetro digitais Minolta (Modelo CR-300, Minolta, Osaka, Japão). Os parâmetros de luminosidade (L^*), vermelho / verde (a^*) e amarelo / azul (b^*) foram determinados de acordo com as especificações do Commission Internationale de L'éclairage (CIE, 1986), sendo: D65 iluminante, 8 ° ângulo de visão e

ângulo observador padrão de 10 ° especular incluído. Duas salsichas de cada tratamento foram analisadas em triplicata.

3.4.2.4. Capacidade de Retenção de Água (CRA)

A capacidade de retenção de umidade realizada nas amostras cozidas foi determinada de acordo com a metodologia descrita por Sayas-Barbera et al. (2011). Para isto, o teor de umidade foi determinado nas amostras antes e depois do tratamento térmico, no qual as salsichas foram cozidas até alcançar 72 °C no centro geométrico, determinado utilizando um termopar (HANNA INSTRUMENTS, HI 935005, Romênia), em seguida foram arrefecidas à temperatura ambiente. A estimativa da quantidade de umidade retida nas amostras foi calculada de acordo com a seguinte equação:

$$\% \text{ Retenção de umidade} = 100 \times \frac{\text{peso cozida (g)} \times \% \text{ umidade na amostra cozida}}{\text{peso cru (g)} \times \% \text{ umidade na amostra crua}}$$

3.4.2.5. Análise do Perfil de Textura

3.4.2.5.1. Análise de Textura 1º e 2º Estudo

As salsichas frescas de cada formulação foram submetidas ao cozimento, depois dos 0, 5, 10 e 15 dias de armazenamento a 4 ± 1 °C e foram analisadas quanto aos parâmetros de textura. Esta análise foi efetuada utilizando texturômetro TA-XT2 (Stable Micro Systems, Haslemere, Inglaterra). As amostras foram cortadas em pedaços de 3 cm de altura. Os parâmetros de textura foram medidos através da compressão das amostras a 25% de sua altura original entre placas planas e uma sonda cilíndrica de 2 cm de diâmetro. As curvas força-tempo foram registradas a uma velocidade de 5 mm / s em uma distância de 35 mm (BOURNE, 1982). Dureza (força de pico do primeiro ciclo de compressão, N), e mastigabilidade (dureza $\times$ coesividade $\times$ elasticidade, N $\times$ mm), coesividade (razão de áreas positivas de segundo ciclo para a área de primeiro ciclo, sem dimensões), gomosidade (dureza $\times$ coesividade, N), elasticidade (distância da altura detectada do produto na segunda compressão dividida pela distância inicial de compressão, mm / mm) e resiliência (área

durante a retirada da primeira compressão dividida pela área da primeira compressão) foram os parâmetros de textura determinados (ANDRES et al., 2006). Duas unidades de cada formulação foram analisadas em duplicado.

3.4.2.5.2. Análise de Textura 3º Estudo

Duas salsichas frescas de cada formulação foram submetidas ao cozimento até atingir 72 °C no centro geométrico após os dias 0, 7, 14 e 21 de armazenamento a 4 ± 1 °C, em seguida foram arrefecidas a temperatura ambiente. Quatro pedaços de 1 cm de altura de cada salsicha foram comprimidos duas vezes utilizando um analisador de textura (Modelo TA.XT plus, Stable Micro Systems, Surrey, England). Uma sonda cilíndrica de 6 mm de diâmetro adaptada com velocidade de 2 mm/s e 70% de compressão foi utilizado para determinar os parâmetros de dureza, elasticidade, coesividade, gomosidade, mastigabilidade e resiliência (LIN; CHAO, 2001).

3.4.3. Análise Sensorial

3.4.3.1. Análise sensorial do 1º Estudo

Para a avaliação sensorial, realizada apenas no Tempo zero (T=0), as salsichas suínas foram submetidas ao cozimento a 130 °C durante cerca de 35 minutos em um forno até atingir uma temperatura interna de 72 °C; em seguida foram cortados em pedaços de 5 centímetros e servidos imediatamente (LIN; HUANG, 2008). O painel sensorial foi composto por nove provadores treinados, sendo estudantes e investigadores de pós-graduação da Escola Superior de Biotecnologia da Universidade Católica Portuguesa, no Porto. As salsichas cozidas foram avaliadas quanto a aparência e sabor, separadamente para cada nível de gordura. A diferença do controle foi o teste usado. Amostras sem quitosana foram usadas como amostras controle. Cada participante recebeu uma amostra rotulada de controle, bem como a amostra controle cego (mesma amostra rotulada de controle) e uma amostra do teste (tratamento adicionado de quitosana). As amostras controle cego e teste foram codificadas com números aleatórios de três dígitos e apresentadas aos provadores em uma ordem equilibrada. Cada participante foi convidado a avaliar a diferença entre as

amostras codificadas e a marcada de controle utilizando a escala fornecida: 0 - mesmo / nenhuma diferença; 3 - diferença moderada e 5 - grande diferença.

3.4.3.2. Análise sensorial do 3º Estudo

Participaram da análise sensorial 18 panelistas treinados que foram esclarecidos dos objetivos da pesquisa através da assinatura do Termo de Consentimento Livre e Esclarecido (Apêndice 1). Para a realização da análise sensorial, foi aplicado o teste da Análise descritiva Quantitativa (ADQ), seguindo-se a metodologia descrita por Faria; Yotsuyanagi (2002) e Ellendersen; Wosiacki (2010). A ADQ envolveu as etapas de recrutamento e pré-seleção de julgadores, desenvolvimento da terminologia descritiva, seleção final dos julgadores e avaliação final das amostras de salsicha.

3.4.3.2.1. Recrutamento e Pré-seleção dos julgadores

Inicialmente, foi enviado um convite via e-mail aos discentes do Curso de Pós-graduação em Ciência e Tecnologia de Alimentos e do Curso de Pós-graduação em Ciências da Nutrição da Universidade Federal do Paraíba. Aqueles com real interesse em participar da avaliação da salsicha inscreveram-se por e-mail, os quais receberam um questionário (Apêndice 2) inquirindo sobre a disponibilidade de tempo, idade, escolaridade, frequência de consumo de carne caprina, derivados cárneos, além de questões relacionadas à saúde. Foram recrutados 25 voluntários.

Na fase de pré-seleção, os provadores foram treinados inicialmente por meio de testes triangulares (Apêndice 3), utilizando salsichas comerciais de frango e de peru e carne caprina e bovina, além de teste de reconhecimento dos gostos básicos (Apêndice 4) e de habilidade com escalas (Apêndice 5). Os provadores que atingiram um mínimo de 70% de acertos foram pré-selecionados (MEILGAARD; CIVILLE; CARR, 1999). Ao final desta etapa foram pré-selecionados 18 julgadores.

3.4.3.2.2. Desenvolvimento da terminologia descritiva das salsichas

O levantamento da terminologia descritiva ou descritores foi realizado através do Método de Rede (MOSKOWITZ, 1983). As três formulações de salsicha caprina foram

servidas aos pares (Figura 4), tais como: salsicha controle, salsicha adicionada de quitosana e salsicha adicionada de quitosana modificada. Os julgadores foram instruídos a expressar as sensações, descrevendo as características percebidas em ordem de aparecimento para cada atributo como aparência, aroma, sabor e textura. Após o levantamento dos termos, a equipe reuniu-se, sob a supervisão do líder, para discutir os termos gerados bem como defini-los. Nessa etapa, os termos que expressaram aspecto sensorial similar foram agrupados em um só atributo. Ao final, foi gerada uma lista de termos descritivos (Apêndice 6), que em reunião com a equipe esses foram definidos e as amostras referências dos extremos (mínimo e máximo) da escala de 9 cm para cada atributo.

3.4.3.2.3. Treinamento e seleção final dos julgadores

As salsichas foram grelhadas em um grill elétrico até alcançar uma temperatura central de 72 °C, monitorado com auxílio de um termopar (HANNA INSTRUMENTS, HI 935005, Romênia) (Figura 5). Após arrefecimento durante 3 minutos, à temperatura ambiente, as amostras foram cortadas em pedaços de 3 cm e servidas para cada provador separadamente (SOULTOS et al., 2008) identificadas com números de 3 dígitos.

O treinamento foi realizado em seis sessões, cada uma com três repetições, para avaliar a reprodutibilidade dos julgadores. As salsichas foram apresentadas junto com as amostras referência (Apêndice 7), as quais foram escolhidas em função dos termos descritivos e sugestões da equipe de julgadores. Foi utilizado uma escala linear não estruturada de nove centímetros ancorada nos pontos extremos, sendo a esquerda pelo termo “pouco”, “fraco” ou “nenhum” e a direita “muito” ou “forte” (ABNT, 1998). Para selecionar a equipe final, os provadores avaliaram as amostras três repetições e os dados foram submetidos à ANOVA com duas fontes de variação, amostra e provador, sendo selecionado para compor a equipe final aquele que teve capacidade discriminatória (p de $F_{amostra} < 0,50$) e reprodutibilidade (p de $F_{repetição} > 0,05$) (DAMÁSIO; COSTELL, 1991).

3.4.3.2.4. Avaliação final das salsichas

Ao final do treinamento foram selecionados 9 julgadores, os quais avaliaram cada uma das amostras de salsicha, monadicamente, em três repetições. A avaliação sensorial foi realizada nos dias 0 e 7 quanto aos atributos de aparência, textura, sabor e aroma. Aos 14

dias as amostras foram avaliadas apenas quanto a aparência e o aroma, pois sensorialmente e microbiologicamente já apresentavam sinais de degradação, não sendo mais avaliadas sensorialmente aos 21 dias de armazenamento. As amplitudes dos atributos foram avaliadas em escalas não estruturadas de 9 cm, com termos de intensidade (pouco/fraco/nenhum e muito/forte) ancorados em seus extremos, sendo utilizado o mesmo modelo de ficha do treinamento (Apêndice 8). Para a consulta foi disponibilizada a tabela de referências, bem como a ficha descritiva, já desenvolvida na etapa de treinamento. Junto às amostras e às referências, foram servidas água e biscoito tipo cracker água e sal para a limpeza do palato e evitar interações entre as amostras (STONE et al., 1974).

3.4.4. Análises Estatísticas

3.4.4.1. Análise estatística do 1º Estudo

O pacote estatístico utilizado foi o software Assistat, versão 7.6 beta (SILVA; AZEVEDO, 2009) para explorar a significância estatística dos resultados. Todos os dados foram avaliados por análise de variância (ANOVA), considerando-se um intervalo de confiança a 95% ($p < 0,05$). Para os resultados das análises físico-químicas e microbiológicas foi utilizado o teste de Tukey com três fatores: tempo de armazenamento, nível de gordura e quitosana. Os dados coletados para a análise sensorial foram avaliados por um teste não-paramétrico para amostras pareadas, utilizando o teste Wilcoxon signed-rank.

3.4.4.2. Análise estatística do 2º Estudo

A análise estatística dos resultados foi realizado usando o pacote estatístico SAS versão 9.3 (SAS, Institute Inc., Cary, NC, 2011). A análise de variância (ANOVA) usando o teste de Tukey foi aplicado para os resultados das análises físico-químicas e microbiológicas para determinar as diferenças estatisticamente significativas entre as formulações durante o armazenamento. Os dados das análises microbiológica, textura e cor foram avaliados pela Análise de Componentes Principais (PCA) e correlação linear de Pearson. Um intervalo de confiança a 95% ($p < 0,05$) foi considerado em todos os casos.

3.4.4.3. Análise estatística do 3º Estudo

Os dados foram analisados usando o pacote estatístico SAS versão 9.3 (SAS, Institute Inc., Cary, NC, 2011). A análise de variância (ANOVA) usando o teste de Tukey foi aplicado para os resultados das análises físico-químicas, microbiológicas e sensoriais para determinar as diferenças estatisticamente significativas entre as formulações durante o armazenamento. Um intervalo de confiança a 95% ($p < 0,05$) foi considerado em todos os casos.

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3. RESULTADOS E DISCUSSÃO

Os resultados obtidos com o desenvolvimento desta pesquisa proporcionaram a elaboração de três artigos:

ARTIGO 2:

Development of a low fat fresh pork sausage based on Chitosan with health claims: impact on the quality, functionality and shelf-life.

Publicado na revista Food & Function, 2015, 6, 2768–2778.

DOI: 10.1039/c5fo00303b

ARTIGO 3:

Goat sausages containing chitosan towards a healthier product: microbiological, physico-chemical textural evaluation

Publicado na revista Food & Function, 2016, 7, 4020–4029.

DOI: 10.1039/c6fo00653a

ARTIGO 4:

Effect of chitosan and chitosan–glucose Maillard reaction product on quality and shelf life of low fat goat meat sausage

Submetido na revista Functional Foods

(ANEXO B)

ARTIGO 2

**DEVELOPMENT OF A LOW FAT FRESH PORK SAUSAGE BASED ON
CHITOSAN WITH HEALTH CLAIMS: IMPACT ON THE QUALITY,
FUNCTIONALITY AND SHELF-LIFE**

PAPER

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Development of a low fat fresh pork sausage based on chitosan with health claims: impact on the quality, functionality and shelf-life

 Deborah S. do Amaral,^a Alejandra Cardelle-Cobas,^b Bárbara M. S. do Nascimento,^a Maria J. Monteiro,^b Marta S. Madruga^a and Maria Manuela E. Pintado^{*b}

A low fat fresh pork sausage based on chitosan was developed with the objective of obtaining a new functional meat product with improved properties and health claims promoting cholesterol reduction. Sausages were formulated with chitosan (2%, w/w) and different fat levels (5%, 12.5% and 20%, w/w). The results indicated that incorporation of 2% chitosan into produced pork sausages with health claims of reduction of cholesterol is technologically feasible. In addition, the chitosan reduced the microbial growth, revealing interesting fat and water absorption capacities, reduced lipid oxidation, provided greater stability in terms of colorimetric parameters and promoted positive firmer texture and gumminess. The reduction of fat content to levels of 5% was positively achieved with the incorporation of chitosan. Sensory analysis showed that panelists did not detect any significant difference in taste and any unfavorable effect on the sausage appearance as a consequence of chitosan addition and variation of fat.

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Introduction

Processed meat products are widely consumed foodstuffs, relatively inexpensive compared to traditional fresh meat cuts.¹ Fresh pork sausages are a meat product, consisting mainly of pork and a variable amount of fat, which are chopped and mixed with water and/or ice and complemented with a variety of non-meat ingredients.² After homogenization, the meat mixture is stuffed into casings and ready-made products are maintained under refrigerated storage conditions until consumption.³ Due to the high fat content, the perishable nature of the raw materials and the lack of thermal processing, such products are prone to spoilage by both lipid oxidation and microbial contamination.⁴ Therefore, several synthetic food additives, such as nitrites, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ), have been used to prevent these harmful events and increase the shelf life of the product.^{1,4} However, nowadays, society is becoming aware of the importance of diet for health. This fact, associated with safety of some synthetic additives, has been questioned in the last few years¹ and has caused an

increasing demand of natural products by the consumers as an alternative to chemical preservatives in foods. Among all the possible additives, chitosan, as a biopolymer with an interesting high antimicrobial capacity, has attracted the attention of the food industry as an alternative to replace the synthetic additives, in order to meet the needs and standards of food safety.^{4–8}

In addition to its antimicrobial capacity, chitosan possesses other interesting properties such as antioxidant capacity,^{4,9} lipid and water binding capacity,^{10–12} and emulsification properties.¹³ Due to these properties, chitosan has been described as an interesting functional and technological ingredient, since it could act not only as an additive, but could also provide improved properties and a better nutritional profile to the final product.¹⁴ Regarding the nutritional and functional benefits, numerous *in vitro* research studies have reported the ability of chitosan to decrease the serum cholesterol.^{15–17} *In vivo* studies in animals have reported that chitosan exhibits hypocholesterolemic and hypolipidemic effects, including the reduction of blood and liver triglycerides (TG) and total cholesterol (TC) levels in animals.^{18–20} Other studies have also reported the hypocholesterolemic effect of chitosan on humans.^{21,22} Recently, due to the consistent evidence on the chitosan capacity to decrease serum cholesterol, the European Food Safety Authority (EFSA) has approved a health claim which establishes that “regular consumption of chitosan contributes to the maintenance of normal blood cholesterol concentrations”. In order to bear the claim, EFSA demands a

^aDEA – Department of Food Engineering, Technology Centre, Federal University of Paraíba, 58051-900 João Pessoa, Paraíba, Brazil

^bCBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquitecto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal.

E-mail: mpintado@porto.ucp.pt; Fax: +351-225090351; Tel: +351 225580097

quantity in food of at least 3 g per day of chitosan in one or more servings.²³ This implies that the functional food is consumed as a part of a balanced diet and on a regular basis, and the selected functional ingredient is integrated into a food matrix with an equilibrated nutritional profile allowing that one or more serving doses ensure the amount required to provide the health claim.

In the context of a healthy diet the demand for low-fat meat products has also increased.²⁴ Lin and Chao²⁵ indicated that chitosan could be used positively into a reduced fat Chinese-style sausage. Their results showed better or similar quality of chitosan sausages regarding physicochemical, microbial and sensory characteristics with no adverse effects on textural properties.

Some studies can be found on the fat reduction in meat products by the incorporation of chitosan,^{9,25} however, to our knowledge, information on the application of chitosan in meat products is not enough to establish whether the products accomplish the specifications regulated by the EFSA to be claimed as functional ingredients contributing to the maintenance of normal blood cholesterol concentrations. Furthermore, most of these studies did not reflect this potentiality.

Therefore, in this study, chitosan was included in an adequate concentration (2%, w/w) to accomplish the EFSA claims (3 g of chitosan per day in one or more servings) on its hypocholesterolemic effects in a low-fat meat matrix with the main objective of establishing whether this inclusion could be technologically feasible and could affect the quality and safety of

the product. The chosen meat matrix was pork sausages. Samples were produced with different percentages of fat with a required amount of chitosan and stored at 4 °C for 15 days. Microbiological, physico-chemical and sensorial aspects were analyzed during the entire shelf-life.

Results and discussion

Microbiological analysis

In the sausages developed in this study, the only added preserving compounds were natural salt, spices and chitosan, with no addition of nitrites or sulphides. Table 1 shows the results obtained for the microbiological counts of the different microbial groups assessed on fresh samples after production and throughout the storage time. In general, it is possible to observe as microbiological counts increased, in all cases, throughout the storage time. In addition, chitosan incorporation induced, in general, a significant reduction of viable cells (*ca.* 0.5–1.0 log CFU mL⁻¹) in the fresh sausage samples, maintaining these differences throughout the storage time. A group of mesophilic bacteria showed at time 0, values ranging from 7.11 ± 0.03 to 8.25 ± 0.03 log CFU g⁻¹ for sausages without chitosan and from 6.80 ± 0.09 to 7.82 ± 0.10 log CFU g⁻¹ for sausages containing chitosan. The presence of high levels of initial mesophilic bacteria is explained by the natural contamination in raw meat, which is dependent on the type of animal, the slicing method and the storage time under

Table 1 Microbial counts (log CFU g⁻¹) obtained for fresh pork sausages with 2% (w/w) of chitosan (1, 2 and 3B) and without chitosan (1, 2 and 3A) prepared with different amounts of fat, 5% (w/w) (sample 1), 12.5% (w/w) (sample 2) and 20% (w/w) (sample 3) and stored at 4 °C for 15 days

Microorganisms	Samples	Storage period ^a (days)			
		0	5	10	15
Mesophilic (log CFU g ⁻¹)	1A	8.25 ± 0.03 ^{CA}	8.32 ± 0.03 ^{bCA}	8.41 ± 0.02 ^{BA}	8.79 ± 0.07 ^{AA}
	1B	7.82 ± 0.10 ^{BB}	7.81 ± 0.09 ^{BC}	7.91 ± 0.06 ^{ABC}	8.00 ± 0.04 ^{AD}
	2A	7.17 ± 0.04 ^{DC}	8.09 ± 0.07 ^{CB}	8.20 ± 0.04 ^{BB}	8.44 ± 0.03 ^{AB}
	2B	6.80 ± 0.09 ^{CE}	7.07 ± 0.05 ^{BD}	7.34 ± 0.03 ^{AD}	7.38 ± 0.03 ^{AE}
	3A	7.11 ± 0.03 ^{CC}	8.14 ± 0.04 ^{BB}	8.23 ± 0.04 ^{abB}	8.25 ± 0.02 ^{AC}
	3B	6.91 ± 0.06 ^{CD}	7.18 ± 0.07 ^{BD}	7.14 ± 0.10 ^{BE}	7.45 ± 0.01 ^{AE}
Psychrophilic (log CFU g ⁻¹)	1A	5.35 ± 0.02 ^{DA}	6.35 ± 0.03 ^{CA}	7.50 ± 0.03 ^{BA}	8.44 ± 0.01 ^{AA}
	1B	5.00 ± 0.07 ^{DC}	5.37 ± 0.02 ^{CD}	7.21 ± 0.02 ^{BC}	7.95 ± 0.04 ^{AD}
	2A	5.23 ± 0.03 ^{DB}	6.13 ± 0.04 ^{CB}	7.3 ± 0.02 ^{BB}	8.35 ± 0.02 ^{AB}
	2B	4.95 ± 0.06 ^{DC}	5.10 ± 0.04 ^{CE}	7.04 ± 0.04 ^{BD}	7.86 ± 0.04 ^{AE}
	3A	5.16 ± 0.04 ^{DB}	5.53 ± 0.01 ^{CC}	7.20 ± 0.02 ^{BC}	8.18 ± 0.04 ^{AC}
	3B	4.81 ± 0.06 ^{DD}	5.01 ± 0.04 ^{CF}	6.89 ± 0.05 ^{BE}	7.79 ± 0.07 ^{AE}
Enterobacteriaceae (log CFU g ⁻¹)	1A	2.80 ± 0.03 ^{DA}	2.94 ± 0.01 ^{CA}	3.87 ± 0.01 ^{BA}	4.65 ± 0.05 ^{AA}
	1B	2.64 ± 0.03 ^{DB}	2.72 ± 0.01 ^{CC}	3.60 ± 0.05 ^{BC}	3.80 ± 0.02 ^{AC}
	2A	2.77 ± 0.04 ^{DA}	2.90 ± 0.02 ^{CA}	3.84 ± 0.03 ^{BA}	4.57 ± 0.03 ^{AB}
	2B	2.54 ± 0.06 ^{DC}	2.66 ± 0.02 ^{CD}	3.55 ± 0.02 ^{BCD}	3.69 ± 0.03 ^{AD}
	3A	2.75 ± 0.01 ^{DA}	2.82 ± 0.01 ^{CB}	3.75 ± 0.03 ^{BB}	4.59 ± 0.03 ^{AB}
	3B	2.56 ± 0.04 ^{CC}	2.59 ± 0.04 ^{CD}	3.48 ± 0.02 ^{BD}	3.73 ± 0.04 ^{AD}
Moulds and yeasts (log CFU g ⁻¹)	1A	5.27 ± 0.03 ^{DA}	6.34 ± 0.04 ^{CA}	6.45 ± 0.03 ^{BA}	7.35 ± 0.03 ^{AA}
	1B	4.90 ± 0.09 ^{CC}	5.99 ± 0.05 ^{BC}	6.09 ± 0.04 ^{BC}	6.95 ± 0.04 ^{AC}
	2A	5.16 ± 0.03 ^{CAB}	6.29 ± 0.06 ^{BA}	6.39 ± 0.04 ^{BA}	7.24 ± 0.05 ^{AB}
	2B	4.85 ± 0.12 ^{DC}	5.84 ± 0.05 ^{CD}	6.06 ± 0.03 ^{BC}	6.87 ± 0.10 ^{AC}
	3A	5.10 ± 0.05 ^{DB}	6.12 ± 0.03 ^{CB}	6.25 ± 0.02 ^{BB}	7.21 ± 0.05 ^{AB}
	3B	4.92 ± 0.10 ^{DC}	5.89 ± 0.08 ^{CD}	6.01 ± 0.04 ^{BC}	6.80 ± 0.07 ^{AD}

^a Different letters (a, b) in the same row differ significantly ($P < 0.05$) in time. Different letters (A, B) in the same column differ significantly ($P < 0.05$) in samples.

refrigeration until use, that in the present work, for pork, it presents high microbial counts. Similar mesophilic count values were reported by Sayas-Barberá *et al.*,¹² which found values of *ca.* 7.0 log CFU g⁻¹ after 8 days of storage in pork model burgers with chitosan. However, these values increased slightly and gradually, with time, the values being always significantly lower in all samples containing chitosan. The values between 8.25 ± 0.02 and 8.79 ± 0.07 in sausage controls and between 7.38 ± 0.03 and 8.00 ± 0.04 log CFU g⁻¹ in sausages with chitosan were found after 15 days of storage at 4 °C.

With regard to the variation of fat ($p < 0.05$), sausages containing chitosan and less fat content (1B) decreased by 0.5 log units at times 0, 5 and 10 days of storage, the reduction being about 0.8 log units after 15 days. In samples 2B and 3B, the reduction was *ca.* 1 log CFU g⁻¹ on days 5, 10 and 15 in relation to the control sample. The antimicrobial effect of chitosan observed in this study is in accordance with that reported by Sayas-Barberá *et al.*,¹² which indicated a reduction of 2 logs in pork model burgers with 1% low molecular weight chitosan at the end of storage (8 days). In fresh pork sausages, Roller *et al.*³⁶ reported a 2 log units reduction after addition of 0.6% (w/w) chitosan in combination with sulfites after 24 days of storage at 4 °C. Georgantelis *et al.*⁴ reported a decrease between 1 and 2 log units in fresh pork sausages with 1% (w/w) chitosan after 20 days of storage at 4 °C. Soultos *et al.*⁷ also indicated that addition of 1% (w/v) chitosan decreased by at least 1 log unit in fresh pork sausages stored for 28 days at 4 °C.

The initial values of psychrophilic bacteria ranged from 4.81 ± 0.06 to 5.35 ± 0.02 log CFU g⁻¹. These values were increasing along the time until reaching the maximum values of 8.44 ± 0.01 log CFU g⁻¹ after 15 days of storage for the samples without chitosan and values of 7.95 ± 0.04 log CFU g⁻¹ for the samples containing chitosan. Regarding the percentage of fat, plausibly the bacterial counts decreased with the increase in fat content and were, also, lower for the samples containing chitosan. The maximal effect of chitosan was produced at 5 days of storage. The data collected in the literature confirm the behavior observed. Thus, Soultos *et al.*⁷ reported the values of *Pseudomonas* (one of the most representative psychrophilic bacteria in food) between 4.07 ± 0.49 and 3.14 ± 0.62 log CFU g⁻¹ for fresh sausages without chitosan and containing 1% (w/w) chitosan, respectively. These values increased after 15 days of storage at 4 °C, reaching the values of 5.68 ± 0.82 and 4.67 ± 0.67 log CFU g⁻¹, respectively. This increase corresponds to 1.5 log CFU g⁻¹ in both samples. After 28 days, the cell counts found were 7.56 ± 0.66 and 6.67 ± 0.56 log CFU g⁻¹, corresponding, in this case, to an increase of 3.5 log units for both samples. In another study with traditional Greek fresh sausages, Georgantelis *et al.*⁴ found the initial values of *Pseudomonas* of about 6.71 ± 0.38 and 5.95 ± 0.30 log CFU g⁻¹ for the samples without and with 1% (w/w) of chitosan, respectively. After 15 days of storage at 4 °C these counts increased until 7.30 ± 0.20 and 6.16 ± 0.36 log CFU g⁻¹, the increase being much lower than that in the case reported

before. However, it is obvious to indicate that the initial counts were also higher.

The group of *Enterobacteriaceae* showed relatively low counts ensuring low contamination of meat, and counts were always lower in samples containing chitosan than in control sausages (without chitosan addition) ($p < 0.05$). Regarding the fat content, the samples containing higher fat amount showed lower microbial counts, but did not differ significantly ($p > 0.05$), however the counts increased significantly throughout the storage time for all samples ($p < 0.05$). García *et al.*³⁷ also corroborated these results in a study on the evaluation of the effect of partial replacement of sodium nitrite in pork sausages by chitosan. These authors reported initial values of *Enterobacteriaceae* *ca.* 1 log CFU g⁻¹ and values greater than 7 log CFU g⁻¹ after 35 days of storage at 4 °C. Georgantelis *et al.*⁴ in a study about the effect on the addition of rosemary extract, chitosan and α -tocopherol in fresh pork sausages found values of 5.01 ± 0.23 and 3.80 ± 0.21 log CFU g⁻¹ in the control sample and in the one containing 1% (w/w) of chitosan, respectively. These values increased during storage at 4 °C reaching values of *ca.* 5.38 ± 0.22 and 3.90 ± 0.17 after 20 days, respectively. Soultos *et al.*⁷ in fresh pork sausages reported initial values between 3.51 ± 0.24 and 2.64 ± 0.11 log CFU g⁻¹ for control samples and samples with chitosan, respectively. These values also increased until 4.90 ± 0.36 and 3.94 ± 0.22 log CFU g⁻¹ after 15 days of storage at 4 °C.

Finally, regarding the counts of yeast and molds, the values obtained for fresh pork sausages at time zero were between 4.85 ± 0.12 and 5.27 ± 0.03 log CFU g⁻¹. During the storage time, these values increased to 6.80 ± 0.07 and 7.35 ± 0.03 log CFU g⁻¹ after 15 days, the increase always being lower in all sausages containing chitosan than in control samples without chitosan. A similar tendency was reported by García *et al.*³⁷ in studies using chitosan for partial substitution of nitrites in pork sausages, who showed an increase of up to 3 log units in the yeast content, while for molds the value was kept constant. Georgantelis *et al.*⁴ found an initial value of 4.90 ± 0.04 log CFU g⁻¹ which increased until 7.93 ± 0.19 after 15 days of storage at 4 °C in samples without chitosan and 6.56 ± 0.08 log CFU g⁻¹ for samples containing 1% (w/v) of chitosan. García Fontán *et al.*³⁸ reported an initial average value for molds and yeasts of *ca.* 4.30 ± 1.73 log CFU g⁻¹ (ranging from 1.60 to 6.99) in samples of “Androlla”. These values also corroborate the initial values found in our study.

Physico-chemical analysis

Proximate composition and pH. The formulation of pork sausage consisted of 2% (w/w) chitosan, this percentage being necessary to meet the requirements of EFSA (3 g per day). Thus, one serving of 3 sausages (150 g containing 3 g of chitosan) a day could contribute to reduce the blood cholesterol level. However, this amount could influence several properties of the sausages, so that proximate composition analysis was performed.

The chemical composition of fresh pork sausages prepared with different fat levels, with and without chitosan, is shown

Table 2 Proximate composition obtained for fresh pork sausages and the water retention capacity (WRC) calculated for raw pork sausages with 2% (w/w) of chitosan (1, 2 and 3B) and without chitosan (1, 2 and 3A) prepared with different amounts of fat, 5% (w/w) (sample 1), 12.5% (w/w) (sample 2) and 20% (w/w) (sample 3) and stored at 4 °C for 15 days

Variables	Samples	Storage period ^a (days)			
		0	5	10	15
Moisture (g per 100 g)	1A	73.10 ± 0.12 ^{aA}	72.21 ± 0.05 ^{bA}	72.13 ± 0.25 ^{bA}	71.73 ± 0.19 ^{bA}
	1B	71.54 ± 0.21 ^{aB}	71.17 ± 0.38 ^{aB}	71.39 ± 0.26 ^{aB}	70.35 ± 0.09 ^{bB}
	2A	69.28 ± 0.16 ^{aC}	69.76 ± 0.16 ^{aC}	68.32 ± 0.30 ^{bC}	66.56 ± 0.08 ^{cC}
	2B	67.21 ± 0.23 ^{aD}	66.16 ± 0.17 ^{bD}	66.31 ± 0.19 ^{bD}	63.90 ± 0.16 ^{cD}
	3A	67.10 ± 0.01 ^{aD}	64.15 ± 0.48 ^{bF}	61.95 ± 0.22 ^{dF}	62.81 ± 0.32 ^{cE}
	3B	66.05 ± 0.33 ^{aE}	64.74 ± 0.37 ^{bE}	62.73 ± 0.27 ^{cE}	61.21 ± 0.18 ^{dF}
Proteins (g per 100 g)	1A	20.09 ± 0.10 ^{aA}	19.13 ± 0.03 ^{bA}	19.64 ± 0.25 ^{aBA}	19.43 ± 0.27 ^{aBA}
	1B	19.57 ± 0.03 ^{aA}	18.87 ± 0.14 ^{aBA}	18.48 ± 0.80 ^{bB}	18.49 ± 0.01 ^{bA}
	2A	19.05 ± 0.03 ^{aB}	18.82 ± 0.22 ^{aA}	18.41 ± 0.24 ^{aB}	18.55 ± 0.14 ^{aA}
	2B	19.25 ± 0.10 ^{aAB}	18.47 ± 0.27 ^{aBA}	18.30 ± 0.65 ^{bB}	17.17 ± 0.36 ^{cB}
	3A	16.34 ± 0.38 ^{aC}	16.02 ± 0.07 ^{aB}	15.83 ± 0.34 ^{aBC}	15.08 ± 0.11 ^{bC}
	3B	17.00 ± 0.46 ^{aC}	15.64 ± 0.10 ^{bB}	15.24 ± 0.03 ^{bCC}	14.55 ± 0.36 ^{cC}
Fat (g per 100 g)	1A	4.76 ± 0.16 ^{bE}	5.10 ± 0.18 ^{aBE}	5.31 ± 0.09 ^{aBE}	6.08 ± 0.17 ^{aE}
	1B	3.58 ± 0.09 ^{bF}	4.30 ± 0.36 ^{aBE}	4.29 ± 0.12 ^{aBE}	4.88 ± 0.59 ^{aF}
	2A	10.52 ± 0.08 ^{cC}	10.85 ± 0.26 ^{cC}	12.15 ± 0.35 ^{bC}	13.19 ± 0.82 ^{aC}
	2B	9.28 ± 0.71 ^{bD}	9.52 ± 0.01 ^{bD}	10.60 ± 0.01 ^{aD}	11.12 ± 0.10 ^{aD}
	3A	18.75 ± 0.23 ^{bA}	19.16 ± 0.55 ^{bA}	20.74 ± 0.65 ^{aA}	21.53 ± 0.54 ^{aA}
	3B	17.38 ± 0.28 ^{bB}	17.33 ± 0.04 ^{bB}	17.75 ± 0.19 ^{bB}	19.24 ± 0.51 ^{aB}
Ash (g per 100 g)	1A	2.27 ± 0.04 ^{bA}	2.27 ± 0.01 ^{bA}	2.40 ± 0.03 ^{bA}	2.71 ± 0.27 ^{aA}
	1B	1.97 ± 0.03 ^{cB}	2.15 ± 0.03 ^{bCAB}	2.20 ± 0.02 ^{bAB}	2.48 ± 0.05 ^{aA}
	2A	1.93 ± 0.07 ^{bBC}	2.08 ± 0.09 ^{aBAB}	2.00 ± 0.04 ^{aBBC}	2.20 ± 0.02 ^{aB}
	2B	1.76 ± 0.15 ^{bBC}	1.99 ± 0.06 ^{aB}	2.11 ± 0.12 ^{aB}	2.16 ± 0.02 ^{aB}
	3A	1.70 ± 0.05 ^{bC}	1.71 ± 0.04 ^{bC}	2.00 ± 0.04 ^{aBC}	2.01 ± 0.03 ^{aB}
	3B	1.88 ± 0.13 ^{bBC}	1.95 ± 0.05 ^{aBB}	1.82 ± 0.06 ^{bC}	2.09 ± 0.23 ^{aB}
WRC (%)	1A	78.59 ± 0.03 ^{cE}	81.67 ± 0.46 ^{bF}	84.21 ± 0.46 ^{aE}	84.19 ± 0.24 ^{aE}
	1B	96.96 ± 0.31 ^{bC}	96.07 ± 0.70 ^{cD}	97.93 ± 0.28 ^{bC}	100.04 ± 0.13 ^{aC}
	2A	91.55 ± 0.40 ^{cD}	89.46 ± 0.67 ^{dE}	95.81 ± 0.36 ^{bD}	97.83 ± 0.11 ^{aD}
	2B	107.59 ± 0.33 ^{cB}	109.60 ± 0.06 ^{bC}	111.26 ± 0.01 ^{aBB}	112.9 ± 0.31 ^{aB}
	3A	97.86 ± 0.43 ^{cC}	116.59 ± 0.20 ^{aB}	112.5 ± 0.23 ^{bB}	112.96 ± 0.62 ^{bB}
	3B	120.19 ± 0.82 ^{cA}	119.01 ± 0.58 ^{cA}	123.30 ± 0.29 ^{bA}	126.34 ± 0.20 ^{aA}

^a Different letters (a, b) in the same row differ significantly ($P < 0.05$) in time. Different letters (A, B) in the same column differ significantly ($P < 0.05$) in samples.

in Table 2. Moisture levels were similar to those reported for fresh pork meat, as the formulation of the meat product was just pork lean.^{39,40} The values obtained for the moisture content were different depending on the percentage of fat and the addition or not of chitosan. Thus, regarding the fat content, it is possible to observe these values being higher for the samples with lower amounts of fat. With respect to chitosan, its addition showed to produce a significant ($p < 0.05$) decrease in the moisture content when compared with the corresponding samples used as a control (no chitosan addition and the same level of fat). This fact is due to the ability of chitosan to absorb water. Sormoli *et al.*⁴¹ evaluated the effect of chitosan hydrogen bonding on lactose crystallinity during spray drying and reported that chitosan can easily absorb moisture by hydrogen bonding with water molecules through its hydroxyl and amine groups.⁴² There is a greater difference between samples 2A and 2B (12.5% (w/w) of fat, with and without chitosan, respectively). A similar behavior was reported by Sayas-Barberá *et al.*,¹² who, in pork model burgers without fat addition, found similar values to those reported in

this work, in samples with less addition of fat. Likewise, they found that the addition of chitosan caused a decrease in the moisture of samples. As can be seen, during storage at 4 °C the moisture content in fresh pork sausages decreased along the time of storage, probably due to the loss of water during storage according to Andrés *et al.*³³ and Soultos *et al.*,⁷ who reported a similar effect in Greek style fresh pork sausages, where they found moisture values ranging 57.4–58.1% (w/w) on day 0 and 54.2–54.7% after 28 days of storage at 4 °C.

Regarding the results obtained for the protein content, the addition of chitosan was not statistically different ($p < 0.05$). The slightly higher values were obtained in sausages with lower fat content, however, these differences were also not significant ($p < 0.05$). Protein levels slightly decreased as a function of the storage time in all samples, however not significant differences were not found ($p < 0.05$). Some authors indicated a possible correlation between protein and lipid oxidation thus reducing the protein content throughout the storage period in pork liver pâté and hamburger.^{43–45}

Analysis of samples showed a lower fat content in samples with chitosan compared with their respective controls without chitosan, being statistically different ($p < 0.05$). This effect is due to the ability of chitosan to bind fat.^{46,47} During storage the fat content increased, which can be explained by the decrease of moisture. This effect was also observed for the ash content, although in this case the increase was slight and there was no statistical difference ($p > 0.05$).

The differences in fat (5, 12.5 and 20% (w/w)) allow for proving that it is possible to produce low fat sausages (5%) with 2% chitosan, ensuring a food matrix with an equilibrated nutritional profile (low fat content) allowing that one serving dose (3 sausages of 50 g) ensures the amount required to provide the health claim.

For pH, samples containing chitosan showed higher values (ranging from 6.68 ± 0.04 to 6.52 ± 0.12) than control samples (5.96 ± 0.02 – 5.86 ± 0.07) (data not shown). Similar behavior was reported by other researchers.^{4,7,12} The increase of pH is due to the basic nature of chitosan¹² promoted by the amino groups present; Sayas-Barberá *et al.*¹² established that the increase in pH values is dose-dependent (6.13 ± 0.06 at 1% (w/w) of chitosan) supporting, thus, our results. The higher values obtained in this study can be a consequence of the major chitosan concentration (2% (w/w)). A significant increase ($p < 0.05$) in values was also observed during the storage period for all samples. In this case, the observed effect can be possibly attributed to microbial proteolysis, which causes protein and amino acid degradation resulting in the accumulation of basic compounds such as ammonia.⁴⁸

Lipid oxidation. Lipid oxidation is one of the most relevant reactions in food chemistry.⁴⁹ The unsaturated fatty acids, especially polyunsaturated fatty acids (PUFA), are highly susceptible to oxidation,⁵⁰ reacting with molecular oxygen *via* a free radical chain mechanism.⁵¹ It contributes to the development of unacceptable organoleptic characteristics and it may also affect the nutritional value or even give rise to toxic compounds in meat and meat products.⁵² Therefore, inclusion of ingredients in the meat formulation could have a significant contribution towards the extension of shelf life.⁴ Sodium nitrite has been widely used for its antioxidant action.⁵³ However, the utilization of nitrite has been limited by this result in the formation of *N*-nitrosamines, a group of compounds that are well known for their carcinogenic and mutagenic activities.⁵⁴ In this respect, chitosan plays an important role since it has been considered as a potential natural antioxidant^{4,9,55} without side effects. In our study, lipid oxidation increased proportionally with the increase of fat, being much more intense in the control samples than in the samples with chitosan (Fig. 1). The most significant values ($p < 0.05$) were observed in samples with 20% (w/w) of fat (3A and 3B), where the presence of chitosan (sample 3B) allowed a decrease in lipid oxidation by 55% at 0 days and by 64% after 15 days of storage at 4 °C, when compared with the control sample (3A). This inhibitory effect is explained by the ability of chitosan to chelate iron ions.⁶ The efficiency of this polymer to control

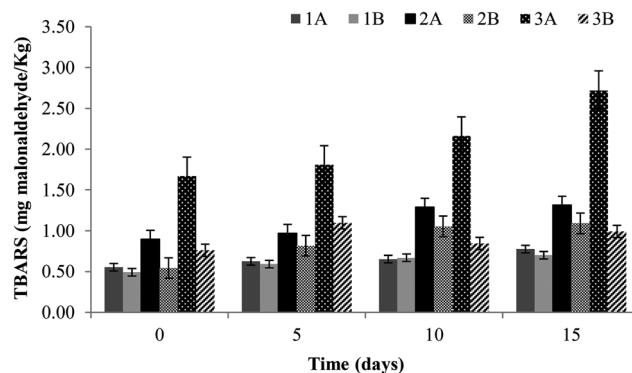


Fig. 1 Evaluation of lipid oxidation in fresh pork sausages with 2% (w/w) of chitosan (samples B) and without chitosan (samples A) prepared with different amounts of fat, 5% (w/w) (sample 1), 12.5% (w/w) (sample 2) and 20% (w/w) (sample 3) and stored at 4 °C for 15 days.

lipid oxidation in meat and meat products has been previously reported by several authors.^{4–7} Oliveira *et al.*¹ reported that the use of natural additives has attracted special attention for presenting antioxidant effects similar to or better than those of synthetic preservatives. In our study, the sample containing 20% (w/w) fat (3B) did not present statistically significant differences ($p > 0.05$) over the 15 days of storage. This behavior was also observed in samples with the lowest fat content (5%), 1A and 1B. In addition, the capacity to reduce efficiently the fat content to values *ca.* 5% with the addition of 2% chitosan ensured a reduced lipid oxidation throughout the storage time, with no significant difference from the control, due to the low fat content. Thus, the addition of 2% (w/w) chitosan or reduction of fat can favour the production of a sausage with better quality and longer shelf life concerning the lipid oxidation profile.

Color measurement. The addition of chitosan significantly affected the color parameters ($p < 0.05$) of fresh pork sausages. In the first days of storage the L^* values were higher for samples with chitosan (Fig. 2a). Kachanechai *et al.*⁵⁶ reported that LMWC can better penetrate the meat matrix due to the smaller size of its granules than HMWC resulting in higher values of L^* , as was observed with our samples. During storage, the L^* value increased in control samples (without chitosan), while in samples containing chitosan this parameter decreased. This difference was more pronounced after 15 days of storage. This increase in the samples without chitosan may be due to the oxidation and concentration of metamyoglobin in the meat. Sayas-Barberá *et al.*¹² found similar results during the storage of fresh pork burgers and justified their results in the same way. Changes in L^* can also be related to surface water, water vapor exchanges between the products and the environment and modifications of the different states of the heme pigments.⁵⁷

The fat content also affected the parameter L^* ($p < 0.05$), since the samples with higher fat content showed higher values of L^* , the highest brightness value being found in

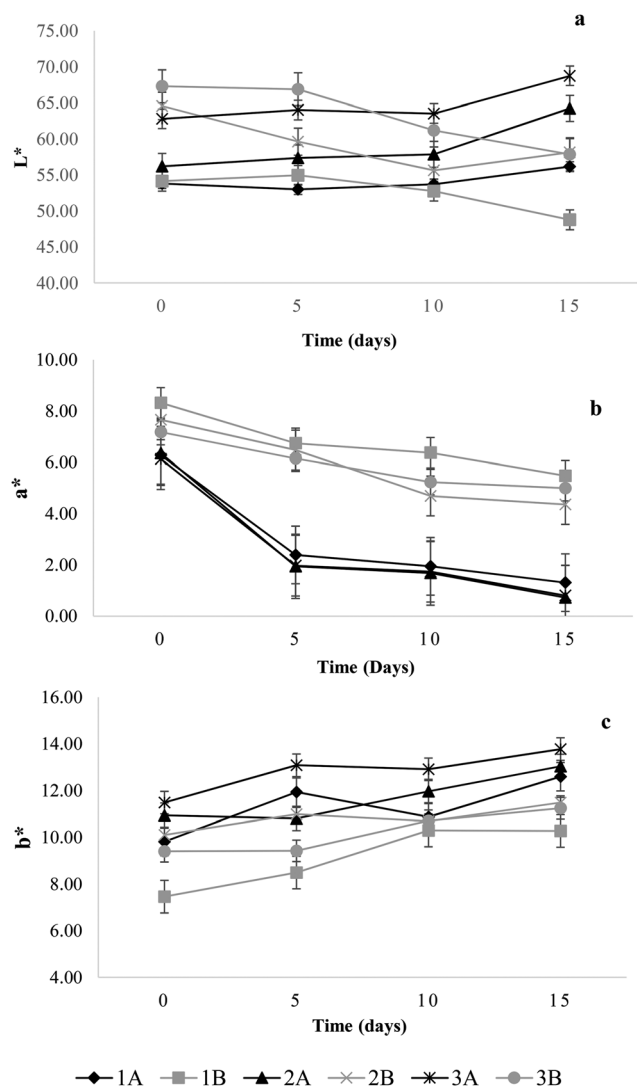


Fig. 2 Evaluation of (a) lightness (L^*), (b) redness/greenness (a^*) and (c) yellowness/blueness (b^*) in fresh pork sausages with 2% (w/w) of chitosan (samples B) and without chitosan (samples A) prepared with different amounts of fat, 5% (w/w) (sample 1), 12.5% (w/w) (sample 2) and 20% (w/w) (sample 3) and stored at 4 °C for 15 days.

samples with 20% of fat. Guerra *et al.*⁵⁸ reported similar behavior in goat mortadella prepared with different levels of fat and goat meat from discarded animals, indicating that a high addition of fat provides a great clarity to the sample.

Regarding the other determined color parameters, in Fig. 2b it is possible to observe as, during the storage period at 4 °C, the values of redness (a^*) decreased for all samples; however, reduction in sausages containing chitosan was lower than that in control samples without chitosan. These differences were statistically significant ($p < 0.05$). This effect may be due to the antioxidant potential of chitosan.^{9,54,59} Youn *et al.*⁶⁰ reported that the addition of chitosan in meat sausages had a more reddish surface than sausages without chitosan. Lee *et al.*⁶¹ investigated the stability of pork meat impregnated

with chitosan solutions (30 and 120 kDa) and concluded that the color of the meat retained its value a^* without changes during storage. The mechanism related to the preservation of the red color can be explained by the chelating properties of chitosan. According to Georgantelis *et al.*,⁴ chitosan could be chelating iron ions of meat hemoproteins during heat processing or storage. Similar behavior was reported by Sayas-Barberá *et al.*¹² who evaluated the effect of concentration and molecular weight in pork model burgers. Regarding the possible influence of fat on the parameter a^* , there was no statistically significant effect ($p > 0.05$) among samples of the same group, however the highest value of a^* was found in samples with 5% (w/w) of fat (1A and 1B). Given these results, we highlight the absence of chemical additives in our study and confirmed that chitosan is a natural antioxidant that has a similar action to nitrites in the preservation of the color red. Song *et al.*⁶² and Soltos *et al.*⁷ reported that the nitrate/nitrite has a long history of use as a precursor in the formation of pink color of cured meats developed for reactions until the formation of the nitrosomyoglobin (NO-Mb) pigment.

The yellow color (b^*) was always lower in samples with chitosan than in control samples (Fig. 2c). During storage, b^* values increased for all samples ($p < 0.05$) indicating that the yellowing of the samples could be related to the intensity of the oxidation process. Fernández-López *et al.*⁵⁷ and García-Esteban *et al.*,⁶³ in a study on the effect of storage time on color properties of pork meat and ham, observed the same trend, and indicated that oxidation could increase b^* values by rancidity. Lin & Chao²⁵ and Sayas-Barberá *et al.*¹² also reported an increase in b^* values with the time of storage.

Water retention capacity. The water retention capacity (Table 2) of cooking pork sausages was affected by the addition of chitosan, by the storage period and variation of fat ($p < 0.05$). The values for control samples (78.55–97.84%) were always lower, at day 0, than the values obtained for samples containing chitosan (95.87–120.01%). The same can be observed after 15 days of storage, the values being 78.59–112.96 and 96.96–126.34 found for the control samples and the samples with chitosan, respectively. Sayas-Barberá *et al.*¹² reported similar behavior and indicated that the highest cooking yield for the hamburger containing 1% (w/w) chitosan (61.90 ± 0.18) than the control samples (58.79 ± 1.30) can be justified by the ability of chitosan to retain water. Thus, the water retention properties of chitosan may be dose dependent, explaining the fact that the values in our study are higher than the values presented by Sayas-Barberá *et al.*¹² since we've used 2% instead of 1%. The ability to hold moisture and other juices before and after heat treatment is one important attribute in sausage and other meat products.⁶⁴ After 15 days, WRC increases for both samples control (1, 2 and 3A) and sausages with chitosan (1, 2 and 3B). Ayadi *et al.*⁶⁵ reported similar behavior in turkey meat sausages with carrageenan and indicated that this increase is probably due to the water loss during storage. Regarding the fat content, the water retention capacity was higher for the samples with higher amounts of fat (3A and 3B). This behavior is consistent with

studies by Cavestany *et al.*,⁶⁶ which reported that higher the percentage of fat, the more concentrated and dense will be the emulsion's continuous phase, favoring, thus, the formation of the structure with greater water-holding ability.

Texture profile analysis. The results obtained from sausage texture analysis are shown in Fig. 3. The increase in hardness and other texture parameters is undesirable, as this effect could have a great impact on consumer acceptability.⁴⁶ The compressive strength of cooked pork sausages was higher in samples containing chitosan, which showed also higher hardness values than control samples without chitosan (Fig. 3a). Lin & Chao,²⁵ García *et al.*⁶⁷ and López-Caballero⁶⁸ reported similar behaviors in studies on the chitosan addition in samples of pork sausages and fish patties, respectively. Kachanechai *et al.*⁵⁶ assessing the influence of chitosan in a model with chicken salt-soluble proteins indicated that the increase in compressive forces resulted in improvement of the texture; this effect is due to the fact that chitosan may act as a binder favoring the formation of a stronger gel. Although addition of chitosan increases the hardness, this is a positive result since a sausage with a more stable structure can be obtained. The stabilization of meat emulsions can be related to the water and

fat holding capacity,⁶⁹ this being the ability of chitosan reported in several studies,^{10,11,47} Furthermore, the hardness increased with reducing fat content and with storage time ($p < 0.05$) in all samples. These effects can be explained with regard to the moisture content, since a loss of water was observed during storage, as described before. Other authors reported the same behavior during storage^{24,32,46} and cooking.⁷⁰

The addition of chitosan in sausages also caused an increase in gumminess values (Fig. 3b) in relation to control samples. These properties showed the tendency to be higher in samples with a lower fat content and a higher time of storage. Estévez *et al.*⁷¹ and López-Caballero *et al.*⁶⁸ indicated that the result of gumminess depends on the hardness, which justifies the similar behaviour shown by these parameters. Hardness is the maximum force required to compress the sample and gumminess is the force necessary to disintegrate a semi-solid state of the sample until swallowing.⁷² The parameters springiness, cohesiveness, chewiness and resilience were not significantly affected by the addition of chitosan in the samples and different percentages of fat ($p > 0.05$) (data not shown). However, the storage time had an effect on resilience, which was slightly reduced ($p < 0.05$) after 15 days of storage.

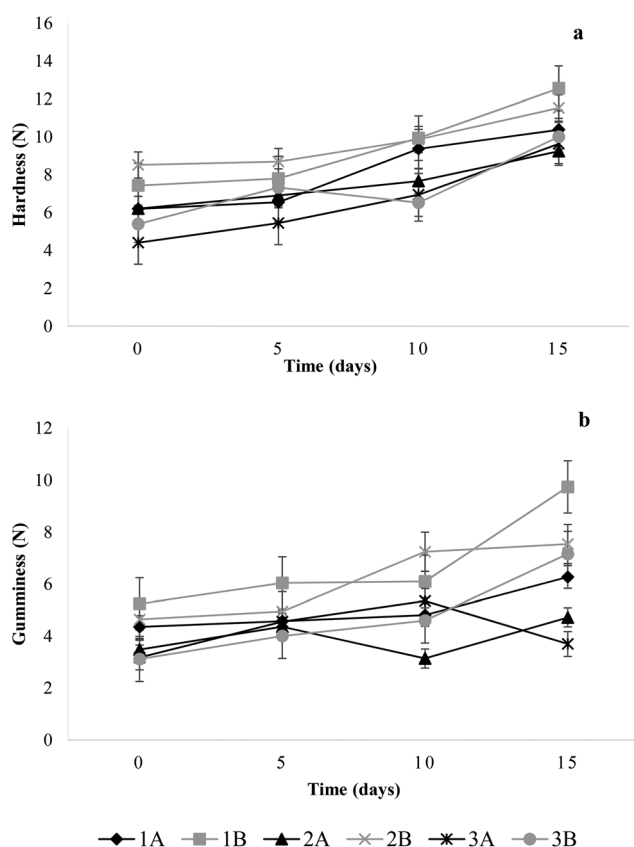


Fig. 3 Evaluation of (a) hardness (N) and (b) gumminess (N) in fresh pork sausages with 2% (w/w) of chitosan (samples B) and without chitosan (samples A) prepared with different amounts of fat, 5% (w/w) (sample 1), 12.5% (w/w) (sample 2) and 20% (w/w) (sample 3) and stored at 4 °C for 15 days.

Sensory evaluation

The sensory scores obtained for pork sausages containing or not containing chitosan and prepared with different levels of fat are shown in Table 3. Regarding the addition of chitosan, the results indicated that no significant differences ($p > 0.05$) were found for the taste due to the addition of chitosan to the products. However, on appearance the samples 1B and 3B (with chitosan) were statistically different ($p < 0.05$) from the respective control samples, 1A and 3A. This effect can be justified mainly by its more intense red color (a^*). Sayas-Barberá *et al.*¹² reported that burgers containing low molecular weight chitosan presented the best visual appearance (pink and shiny), which is in concordance with the data shown here. Soultos *et al.*⁷ also reported that sausages prepared with chitosan scored slightly higher in appearance than the respective

Table 3 Sensory evaluation obtained for fresh pork sausages with 2% of chitosan (1, 2 and 3B) and without chitosan (1, 2 and 3A) prepared with different amounts of fat, 5% (w/w) (sample 1), 12.5% (w/w) (sample 2) and 20% (w/w) (sample 3)

Samples	Appearance ^a	Taste ^a
1A	0.78 ^b	0.56 ^a
1B	2.56 ^a	1.78 ^a
2A	0.89 ^b	1.22 ^a
2B	1.56 ^a	2.11 ^a
3A	0.78 ^b	1.33 ^a
3B	2.33 ^a	2.22 ^a

^a Paired comparisons performed within the same fat content level. Different superscripts indicate no significant differences ($p > 0.05$).

control sample, but statistical significance was not found after the first 7 days of storage. Lin and Chao²⁵ indicated that chitosan did not cause a negative effect on the flavor and no significant off-odor was noted after cooking in sausage. In general, the addition of 2% chitosan on sausages resulted in a moderate difference in appearance compared with the control sausages (without chitosan), both being similar in the taste.

The obtained results give a positive result since no differences were found among samples containing or not containing chitosan and taking this into account, in the literature, it is possible to find a lot of references on the perception of astringency in other food matrices treated with chitosan.^{73–75} Rodríguez *et al.*⁷⁶ reported a high correlation among the astringency intensities which increased when the solution pH decreased. Thus, meat matrices due to their pH values near neutrality can reduce the perception of astringency of chitosan, allowing the development of functional foods without changing the taste significantly.

Experimental

Sausage ingredients and chitosan

The raw meat (pork meat and fat), ingredients (salt, fresh garlic, white pepper powder and dried oregano) and artificial casings were obtained from local markets in the city of Porto (Portugal). Low Molecular Weight Chitosan (LMWC, Sigma-Aldrich, Steinheim, Germany), previously characterized,²⁶ with a Molecular Weight (M_w) of 135 kDa and 90% deacetylated, was used in the study.

Fresh sausage manufacture

An equilibrated fresh sausage formulation was previously designed and consisted of 77% (w/w) of minced pork meat, 10% (v/w) of water, 1.5% (w/w) of salt, 1.3% (w/w) of fresh garlic, 0.2% (w/w) of white pepper powder and 0.1% (w/w) of dried oregano.

The chitosan content was selected in order to be sufficiently high to meet the requirements of the recently passed EFSA health claim (3 g per day). So, chitosan was added at a concentration of 2% (w/w) in order to ensure that in one serving of sausages (150 g, corresponding to 3 sausages of ca. 50 g containing 1 g of chitosan each), the consumption of 3 g of chitosan is ensured. Namely, an intake of these sausages, according to the nutritional recommendation, goes along with a chitosan intake that is sufficiently high to have health promoting effects.

Pork fat was added at different concentrations: 5% (w/w) (Formulation 1B), 12.5% (w/w) (Formulation 2B) and 20% (w/w) (Formulation 3B). A sample without chitosan was used as a control for each formulation (1A, 2A and 3A). Preparation of sausages was carried out following typical procedures for the preparation of this kind of product. Thus, minced pork meat was mixed with the corresponding amount of fat. Then, the rest of the ingredients were added consecutively one by one, chitosan being added at last. All the ingredients were fully

homogenized manually for 5–10 min. After homogenization, the mixture was embedded in artificial casings obtaining fresh sausages with 3 cm diameter and 50 g per unit. Pork sausages were packed in plastic bags without vacuum and stored under refrigeration at 4 °C for 15 days. One lot of 1000 g of fresh pork sausage of each formulation was prepared and divided into two replicates, which were analyzed in duplicate.

Microbiological analysis

With the objective to evaluate the microbiological quality throughout the storage time of the fresh pork sausages, mesophilic and psychrophilic bacteria as well as *Enterobacteriaceae* and yeast and mold counts were analysed at 0, 5, 10 and 15 days of storage at 4 °C. Thus, 8 g of sample in 80 mL of sterile peptone water were placed in plastic bags and homogenized for 2 min in a Stomacher (Lab Blender 400, London, UK). The homogenate was serially diluted with sterile peptone water and viable counts were assessed by the drop method (20 µL of each dilution), as described by Miles *et al.*²⁷ except for *Enterobacteriaceae*, in which the pour plate technique was used. Specific medium and incubation conditions for each microorganism were used. Thus, plate count agar (PCA, Biokar diagnostics) was used for mesophilic and psychrophilic bacteria, and the plates were incubated at 30 °C for 48 h and 7 °C for 7 days, respectively. Yeasts and molds were grown on Potato Dextrose Agar (PDA, Biokar Diagnostics), the plates being incubated at 25 °C for 5 days. *Enterobacteriaceae* were grown in Violet Red Bile Glucose Agar (VRBGA, Lab) and the corresponding plates were incubated at 37 °C for 24 h.

After incubation, the colonies were enumerated and the colony forming units (CFU mL⁻¹) were calculated.

Physicochemical analyses

Moisture, protein, fat, ash and pH. Moisture, ash, protein, and fat contents of samples were determined, in raw sausages, at 0, 5, 10 and 15 days of storage at 4 °C by the official AOAC methods of analysis as 24.003, 24.009, 24.027, and 24.005, respectively.²⁸ In brief, the methodology is described as follows.

Moisture (g water per 100 g sample) was determined by drying the samples at 105 °C to constant weight. The ash content (g ash per 100 g sample) was calculated after incineration of the samples in a muffle at 550 °C and weighed. Proteins (expressed as g protein per 100 g sample) were analyzed by the Kjeldahl method. The fat content (g fat per 100 g sample) was calculated by weight loss after extraction with hexane using Soxhlet apparatus.

The pH values of the samples were also measured by an AOC method of analysis. Specifically, they were analyzed by the 943.02 method.²⁹ A combined pH glass electrode connected to a pH-meter MicropH 2001 Crison Potentiometer (MicropH 2001, Barcelona, Spain) was used.

Lipid oxidation. Lipid oxidation was assessed by measuring the thiobarbituric acid reactive substances (TBARS) by the method adapted from ref. 30. Thus, 2 g of sample were homogenized by vortexing in 10 mL of 10% (v/v) of trichloroacetic

acid (TCA biochemical/Applichem) and 5 ml of 0.02 M 2-thio-barbituric acid (TBA, Merck). Then, it was centrifuged at 5000 rpm for 20 min in a Universal 320R centrifuge (Zentrifugen, Hettich). The supernatant was collected and filtered, heated in boiling water for 35 min at 100 °C and chilled in ice water for 10 min. Finally, the absorbance at 532 nm was measured using a UV mini 1240 spectrophotometer (Shimadzu, Tokyo, Japan). 1,1,3,3-Tetraethoxypropane (Sigma Aldrich) was used as the standard in the range 1×10^{-6} – 14×10^{-6} mol L⁻¹. TBARS concentration was expressed as mg malondialdehyde per kg of sample. Each of the replicated fresh pork sausages was analysed in duplicate.

Color analysis. The color of each kind of sausage sample was determined according to the methodology described by Abularach *et al.*,³¹ using a digital Minolta Colorimeter (Model CR-300, Minolta, Osaka, Japan). The parameters such as lightness (L^*), redness/greenness (a^*) and yellowness/blueness (b^*) were determined under the conditions indicated below, according to the specifications of the Commission Internationale de L'éclairage (CIE, 1986): illuminant D65, 8° viewing angle and a standard observer angle of 10° specular included. Determination in each of the replicated fresh pork sausage samples was performed in triplicate.

Analysis of cooked samples

It is important to highlight that sausages are consumed after cooking and that some parameters in cooked products enable us to understand how chitosan incorporation is affecting the sausages. Since chitosan impacts especially the fat and water features, moisture retention and some textural parameters were analyzed in cooked samples.

Moisture retention after cooking. The estimation of moisture retention in the sausage samples was determined according to the methodology described by Sayas-Barberá *et al.*¹² Thus, the sausages were cooked in an oven at 150 °C to a core temperature of 72 °C. This internal value of temperature was determined at the geometrical center of the samples by inserting a thermocouple. After cooking, sausages were maintained at room temperature until cooling. The samples were weighed and measured before and after cooking. The estimation of the amount of moisture retained in the samples was calculated according to the following eqn (1):

% Moisture retention

$$= \frac{100 \times \text{cooked weight(g)} \times \% \text{ moisture in cooked sample}}{\text{raw weight(g)} \times \% \text{ moisture in raw sample}} \quad (1)$$

Texture profile analysis (TPA). Fresh sausages of each formulation were subjected to cooking after 0, 7, 5, 10 and 15 days of storage at 4 °C and analysed in terms of texture. This analysis was carried out using a texture analyzer TA-XT2 (Stable Micro Systems, Haslemere, England). The samples were cut into pieces of 3 cm height. The textural parameters were measured by compressing the samples to 25% of their original

height between flat plates and a cylindrical probe with a cylinder probe of 2 cm diameter. Force–time curves were recorded at a crosshead speed of 5 mm s⁻¹ at a distance of 35 mm.³² Hardness (peak force of the first compression cycle, N), chewiness (hardness $\times$ cohesiveness $\times$ springiness, N $\times$ mm), cohesiveness (the ratio of positive areas of the second cycle to the area of the first cycle, dimensionless), gumminess (hardness $\times$ cohesiveness, N), springiness (the distance of the detected height of the product on the second compression divided by the original compression distance, mm mm⁻¹) and resilience (area during the withdrawal of the first compression divided by the area of the first compression) were the textural parameters determined.³³ Two units of each formulation were analysed in duplicate.

Sensory analysis

For sensory evaluation, performed only on day 0, the sausages were subjected to 130 °C for about 35 minutes in an oven to reach an internal temperature of 72 °C, and then were subsequently cut into 5 cm pieces to be served immediately.³⁴ The sensory panel was composed of nine trained panelists selected from graduate students of the School of Biotechnology, Catholic University of Portugal in Porto. The cooked pork sausages were evaluated for appearance and taste, separately for each level of the fat content. A difference from the control test was used. The samples without chitosan were used as control samples. Each panelist received a labeled control sample, a blind control sample plus a test sample. The blind control and test samples were coded with three-digit random numbers, and presented to panelists in a balanced order. Each panelist was asked to rate the difference between the coded samples and the labeled control using the provided scale: 0 – same/no difference; 3 – moderate difference and 5 – big difference.

All experiments were performed in accordance with the relevant laws and guidelines for sensory testing of food products. All food ingredients were obtained *via* commercial suppliers and all additives were food-grade. Preparation prior to testing was performed in a dedicated preparation kitchen by trained food technologists. Sensory evaluation experiments that do not involve testing on under 16 year olds or the inclusion of alcohol are approved by the Board of Directors of the CBQF Research Centre. An informed written consent was obtained prior to the experiment.

Statistical analysis

The statistical package used was the Assistat software, version 7.6 beta³⁵ to explore the statistical significance of the results. All data were evaluated by analysis of variance (ANOVA), considering a confidence interval at the 95% level ($p < 0.05$). For the results of physicochemical and microbiological analyses the Tukey test was used with three factors: storage time, level of fat and chitosan. Data collected for the sensory analysis were evaluated by a non-parametric test for paired samples using the Wilcoxon signed-rank test.

Conclusions

The results herein obtained indicated that incorporation of 2% chitosan (corresponding to 1 g chitosan per sausage) into pork sausages to ensure the ingestion of 3 g of chitosan per day (3 sausages) and to accomplish, thus, the EFSA claims of reduction of cholesterol is technologically feasible and also allows us to obtain a product with improved properties, namely if fat reduction is sought. Besides the functional value, the results also indicate that chitosan possesses interesting potential to be included in fresh pork sausages, since it causes an increase in the stability and shelf-life of the product, considering the reduction of microbial growth and lipid oxidation. It also promotes a best red color, a more stable emulsion, by the ability to bind water and fat, and a firmer texture by increase of compressive forces, without negatively affecting the sensory properties. Thus, the results here indicate that although it is necessary to conduct further studies, the addition of chitosan, besides the generation of a functional product with health claims can act positively on the quality and shelf-life of pork sausages, and enable efficiently the reduction of the fat content.

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ARTIGO 3

**GOAT SAUSAGES CONTAINING CHITOSAN TOWARDS A HEALTHIER
PRODUCT: MICROBIOLOGICAL, PHYSICO - CHEMICAL TEXTURAL
EVALUATION**

PAPER

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Goat sausages containing chitosan towards a healthier product: microbiological, physico-chemical textural evaluation†

Deborah S. do Amaral,^a Alejandra Cardelle-Cobas,^{‡b} Bárbara M. S. do Nascimento,^a Marta S. Madruga^a and Maria Manuela E. Pintado^{*b}

Goat meat is extensively known for its interesting nutritional value and for being an important source of protein with high quality. Its food derivatives are, therefore, a good alternative to develop new products addressed to health conscious consumers. In this work, a healthier goat product, namely, a low fat fresh sausage, was produced with the objective of evaluating the effect of inclusion of chitosan on quality, stability and shelf life. Sausages containing 2% chitosan were formulated with different fat levels (5%, 12.5% and 20%, w/w) and stored at 4 °C for 15 days. Results indicated the incorporation of 2% (w/w) chitosan was technologically feasible, due to the reduction of microbial growth and lipid oxidation, as well as the enhancement of red color. Additionally, the treated samples improved all the characteristics associated with cooking, showing the ability to bind water and fat and acquiring a firmer texture compared with control samples. Additionally, the reduction of fat content is technologically feasible without negative influences on the final product.

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Introduction

Goats are ruminants widely distributed around the world and have been a source of nutrients for humans since the very beginnings of civilization.¹ Goat meat has great potential in merchandising because of its nutritional value, such as its low fat content, high digestibility, high protein content, iron and unsaturated fatty acids, when compared to other types of red meat.² The best cuts (loin, leg, *etc.*) are sold at high prices and are very appreciated by the consumers, whereas the remaining cuts such as the shoulder and neck, for example, have low consumer acceptability and no commercial value. In addition to the low appeal for some cuts, consumers reject, in some cases, goat meat probably due to the texture and strong flavour, particularly present in meat from older animals.¹ In this sense, the industrialization of this kind of meat, focusing, specially,

in the elaboration of new products from the less appreciated cuts, could be a good alternative to increase the value of goat meat with low commercial attractiveness.³

Sausage is a popular processed meat product that traditionally consists of chopped meat, water, binders, fat, and seasonings.⁴ Despite its popularity, this kind of product presents some negative perceptions, most of them related to its high fat content, since it is well known that the amount and type of fat consumed is associated with the risk of coronary heart disease.⁵ This, combined with the fact that the effect of diet on health is well-known and that the use of foods to improve health and state of wellbeing is an idea increasingly accepted by society in the last three decades, has caused an increase in the demand for low fat products. Consequently, this change in consumer mentality could contribute to the loss of market share, especially in this kind of product with high fat content such as sausages, when health considerations are important quality criteria.⁶ Another negative aspect usually associated with this kind of product such as sausages, especially when commercialized as a fresh product, is the frequent use of high levels of synthetic additives to avoid damaging lipid oxidation reactions, controlling the growth of pathogens and food contaminants and thus, increasing shelf life.⁷ Therefore, a reformulation of meat products based on processing strategies is an important trend to develop products that promote better consumer health. In this sense, the safety of synthetic additives has been questioned in the last few years, increasing the

^aDEA – Department of Food Engineering, Technology Centre, Federal University of Paraíba, 58051-900 João Pessoa, Paraíba, Brazil

^bCBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquitecto Lobo Vital, Apartado 2511, 4202-401 Porto, Portugal.

E-mail: mpintado@porto.ucp.pt; Fax: +351-225090351; Tel: +351 225580097

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[‡]Present address: Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Veterinary Medicine, University of Santiago de Compostela, 27002 Lugo, Spain.

request for natural additives.⁸ In this sense, different herb extracts, such as for example rosemary, essential oils, as well as some antioxidant compounds, such as α -tocopherol, present in fruits and vegetables have been tested as natural additives, giving rise to interesting results in terms of food preservation.⁷ The use of nitrite and nitrate in the manufacturing of meat products is considered indispensable, since they can promote the red colour of cured products, act as antioxidants by delaying lipid oxidation, prevent or retard microbial growth and give a pleasant flavour.⁹ However, nitrite also results in the formation of *N*-nitrosamines, a group of compounds that are well known for their carcinogenic and mutagenic activities¹⁰ being related to certain types of cancer such as colon cancer. These risks have caused a rise in consumer demand for natural products and created a need for developing alternative preservation systems for meat and meat products.^{11,12}

In this context, different compounds obtained from natural sources such as grains, oilseeds, spices, fruit and vegetables have been investigated.⁸ Among these different natural compounds, chitosan has attracted especial interest from industry as a potential natural food preservative since it exhibits strong antimicrobial activity against a range of food-borne microorganisms¹³ and possesses different functional characteristics, such as antioxidant activity⁷ and lipid and water binding capacity¹⁴ that can promote the final quality of food products. At the same time, different studies have reported several health benefits, namely, anti-inflammatory, antitumoral and immune-stimulating ones.¹⁵ Moreover, many studies report their hypocholesterolemic effects.^{15,16}

Chitosan consists of polymeric 1,4-linked 2-amino-2-deoxy- β -D-glucose and it was reported as Generally Recognised as Safe (GRAS) by the US FDA.¹⁷ Previous studies have indicated that chitosan could be effectively used to inhibit microbial spoilage and delay lipid oxidation in fresh pork sausages at certain concentrations,^{5,7} as well as some research can be found on fat reduction in meat products by the incorporation of chitosan without causing adverse effects in sensory characteristics and textural properties.^{5,18} Moreover, a previous study by our research team indicated that incorporation of 2% (w/w) of chitosan in pork sausages to accomplish the EFSA¹⁹ claims on reduction of cholesterol (ingestion of 3 g of chitosan per day (3 sausages)), is technologically feasible, allowing to obtain a product with improved properties.¹⁸ However, the application of chitosan in goat meat products demonstrating the potential of chitosan as a functional ingredient and as a technological agent to develop a low fat goat meat product with better quality and stability throughout the shelf-life have not been yet established. Additionally, the use of goat meat cuts with low demand could enhance their value and promote the use of this very healthy kind of meat. Therefore, the objective of this study was to include chitosan in an adequate concentration to be in accordance with the scientific opinion of EFSA¹⁹ in a fresh low fat goat sausage and establish whether this inclusion could affect the quality, stability and shelf life of the product. Thus, the fresh goat sausages were produced with different percentages of fat with the required amount of

chitosan and stored at 4 °C for 15 days to evaluate the microbiological and physico-chemical parameters in order to promote goat meat with commercial value through the reduction of fat and use of a natural preservative.

Results and discussion

Fresh samples analysis

Microbiological results. Results on microbiological counts of total mesophilic and psychrotrophic bacteria, *Enterobacteriaceae* and moulds and yeasts in fresh goat sausages during refrigerated storage are shown in Fig. 1. In general, the enumerations for all determined microbiological groups were significantly ($p < 0.05$) affected by storage time, addition of chitosan and variation of fat. In the beginning (day 0) mesophilic bacterial counts (Fig. 1a) were between 5.03 ± 0.07 and 5.24 ± 0.06 for the sausage control and 3.86 ± 0.06 to 4.83 ± 0.07 for the sausages with chitosan, which denotes the efficient and rapid antimicrobial effect of chitosan. These values are in agreement with previously published results²⁰ for fresh goat meat ($5.68 \log \text{cfu g}^{-1}$) and goat meat salted for 0.6 day of salting per kg (4.17 and $3.30 \log \text{cfu g}^{-1}$ for samples without and with olive oil and paprika, respectively). During storage the counts increased gradually in all samples as expected due to the initial microbial load; however, the growth was always higher in the control sausage and on day 15 the counts for control sausages (8.42 ± 0.03 to 7.37 ± 0.02) were very high exceeding the recommended limit of 10^7cfu mL^{-1} ,¹² while in the treated samples with chitosan the initial reduction of ca. 2 log cycles was maintained ensuring that the value is in the recommended range (6.14 ± 0.06 to 6.97 ± 0.06). Similar behavior was reported by Soutos *et al.*²¹ which reported that after storage at 4 °C of Greek style fresh pork sausages for 14 days the total counts in the samples without chitosan (7.82 ± 0.59) had already exceeded the maximum levels acceptable (10^7cfu g^{-1}) of mesophilic bacteria, while samples with chitosan reached 21 days (6.82 ± 0.48). In our study, considering the counts of mesophilic bacteria, the shelf life of the control sample could be limited to 10 days, while the chitosan sausages could prolong at least in 5 days the shelf-life of the treated samples.

Regarding the variation of fat, the bacterial counts decreased with the increase in fat content, being always lower in all sausages containing chitosan where the differences were between 0.5–2 log cycles in relation to the control sample, varying with the level of fat, but with no relation established. In general, the increase of fat led to a reduction in microbial growth explained by the reduction of moisture and the negative effect of fat. Additionally, the inhibition of mesophilic bacteria by chitosan is also in agreement with results reported by other research groups for pork meat products. Other authors⁷ observed a decrease between 1 and 2 log cycles in pork sausage added 1% chitosan after 20 days of storage, while Roller *et al.*²² and Sayas-Barberá *et al.*¹² reported a reduction of 2 log units in fresh pork sausage with 1% chitosan after 24 days and in pork model burgers after 7 days, respectively.

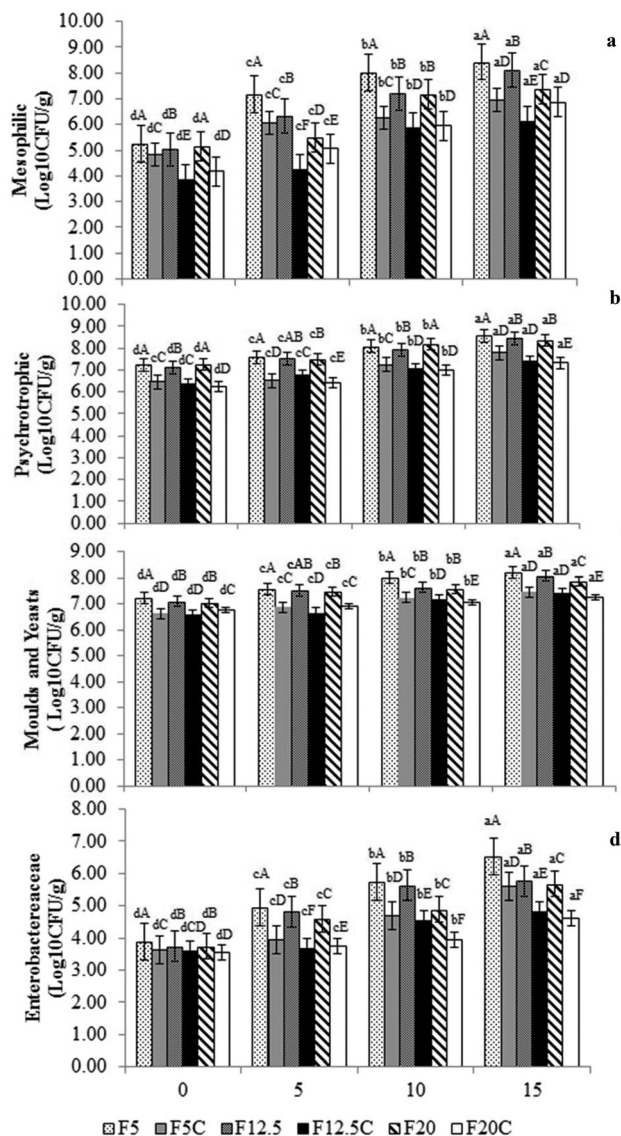


Fig. 1 Evolution of mesophilic (a) and psychrotrophic (b) bacteria, *Enterobacteriaceae* (c) and moulds and yeasts (d) in fresh goat sausages prepared with different amounts of fat, 5%, 12.5% and 20% and with 2% chitosan (samples F5C, F12.5C and F20C) and without chitosan (samples F5, F12.5 and F20) stored at 4 °C for 15 days. Different letters (A, B) differ significantly ($p < 0.05$) in time. Different letters (a, b) differ significantly ($p < 0.05$) in samples. A confidence interval of 95% ($p < 0.05$) was considered in all cases.

The psychrophilic bacterial counts (Fig. 1b) in the early days of storage were between 6.24 ± 0.02 and 7.27 ± 0.03 log cfu g⁻¹. During storage, the counts increased up to values of 8.35 ± 0.02 to 8.58 ± 0.01 log cfu g⁻¹ after 15 days in control samples (without chitosan) and values of 7.32 ± 0.04 to 7.79 ± 0.06 log cfu g⁻¹ for samples with chitosan. Similar counts were found in goat meat under aerobic storage (9 log cfu g⁻¹ after 28 days) and vacuum storage (7 log cfu g⁻¹ after 40 days).²³ Regarding the percentage of fat, the psychrophilic bacterial counts showed a slight decrease, at 10 and 15 days of storage, with the increase in fat content. Psychrophilic

bacterial counts were always lower for the samples containing chitosan and over the entire storage period, showing a reduction in the range of ca. 0.8–1.0 log cfu g⁻¹ in comparison with samples without chitosan. Petrou *et al.*²⁴ reported that chitosan produced significantly lower psychrotrophic counts as compared to the control samples (day-12). Similarly Bostan *et al.*²⁵ reported that during storage the increase in the number of psychrotrophic bacteria in sausage treated with 1% chitosan was significantly lower than the control sausage.

The values of *Enterobacteriaceae* (Fig. 1c) found on day 0 for the sausages with and without chitosan were between 3.54 ± 0.02 and 3.87 ± 0.02 , respectively. These values increased during storage at 4 °C reaching values of ca. 4.72 ± 0.04 and 6.53 ± 0.02 for the sausages with and without chitosan, respectively. Babji *et al.*²³ reported similar counts in goat meat under aerobic storage (6 log cfu g⁻¹) and vacuum storage (4 log cfu g⁻¹) after 28 days. Regarding the fat content, the samples containing higher fat levels (F20 and F20C) also showed significantly lower values of *Enterobacteriaceae*. The addition of chitosan caused a reduction of 1 log cfu g⁻¹ at 5, 10 and 15 days as compared to the control samples. The antimicrobial effect of chitosan upon *Enterobacteriaceae* observed in this study is in accordance with Petrou *et al.*²⁴ which reported that in chicken meat *Enterobacteriaceae* reached final counts of ca. 6 log cycles on day 12, while the treated samples with chitosan showed counts of ca. 3–4 log cycles. Georgantelis *et al.*⁷ studied the effect of rosemary extract addition, with chitosan and α -tocopherol in fresh pork sausages and reported a decreasing trend on *Enterobacteriaceae* counts in samples containing chitosan (3.9 log cfu g⁻¹) and a reduction during storage for 20 days of ca. 1–1.5 log cfu g⁻¹ compared to the control sample.

Finally, with regard to moulds and yeasts (Fig. 1d) fresh goat sausage counts at the initial time were between 6.62 ± 0.01 and 7.24 ± 0.02 log cfu g⁻¹, respectively for sausages with and without chitosan. These values increased during the storage up to 7.25 ± 0.02 and 8.21 ± 0.03 log cfu g⁻¹, respectively after 15 days storage at 4 °C. The lowest levels were always found lower in samples with chitosan (F5C, F12.5C and F20C), in comparison with those with no inclusion of chitosan. The presence of chitosan promoted a reduction in moulds and yeast counts of ca. 0.5–0.8 log cfu g⁻¹ during storage as compared to the control samples. Soutos *et al.*²¹ reported similar results for yeast and molds. They determined that the counts in fresh pork sausage reduced approximately 0.8 log cfu g⁻¹ at the end of 15 storage days at 4 °C when in the presence of 1% of chitosan. Sagoo *et al.*¹¹ in chilled pork products found a reduction of ca. 2 log cfu g⁻¹ at the end of 18 days storage in the presence of 1% chitosan. Petrou *et al.*²⁴ also reported that chitosan could reduce the growth of these species as compared to untreated samples, therefore, corroborating the results found in our study.

Physico-chemical analysis

Proximate composition and pH. Proximate composition of fresh goat sausages with chitosan and prepared with different

pounds as thiobarbituric acid reactive substances (TBARS) as for example malondialdehyde (MDA), as well as, affects sensory traits of meat product, causing flavor, color and texture deterioration.²⁷

The results of TBARS expressed as mg of MDA per kg (see the ESI†) content in fresh goat sausages with and without chitosan prepared with different fat levels during the 15 d of storage increased proportionally with the increasing fat and storage period, lipid oxidation being more intense in the control samples than in the samples with added chitosan. The reduction in the TBARS values was greater in samples with 12.5 and 20% of fat treated with chitosan (F12.5C and F20C), resulting in decreases of *ca.* 44% and 50% on day 0 and 25% and 20% after 15 days, respectively, compared to the control sample with the same fat content. Our results confirm the fact that chitosan may retard oxidative rancidity in muscle foods, by acting as a chelator on transition metal ions, such as ferrous ions.³¹ The effectiveness of chitosan on the oxidative stability of meat and meat products has already been demonstrated.^{7,18,21} Thus, the addition of chitosan can result in better quality and longer shelf life concerning the lipid oxidation profile either in pork or goat meat products.

Color measurement. Changes in color parameters L^* , a^* and b^* are shown in Table 2. The results obtained for samples on day 0 were similar to those reported by Guerra *et al.*³² for goat mortadella prepared with different levels of fat (10%, 20% and 30%). The addition of chitosan resulted in the lowest L^* values, while control samples (without chitosan) had significantly higher values ($p < 0.05$). Sayas-Barberá *et al.*¹² reported similar results in pork model burgers, indicating that the increase in L^* during the first day could be related to oxidation increasing metmyoglobin concentration. During storage, the lightness (L^*) of fresh goat sausages decreased significantly ($p < 0.05$), remaining lower in samples with added chitosan when compared with the corresponding samples used as a control (no chitosan addition and the same level of fat). This decrease in samples F5C, F12.5C and F20C could be due to the water binding ability of chitosan, being in accordance with Fernández-López *et al.*³³ which indicated that the increase in water holding capacity (WHC) reduces L^* . Regarding the fat content, the lightness increased proportionally with the addition of chitosan ($p < 0.05$), so the higher L^* values were found in samples with 20% fat. Similar behavior was reported by Guerra *et al.*³² in goat mortadella prepared with different levels of fat, indicating that higher added fat provides greater clarity to the sample.

The redness (a^* values) of fresh goat sausage decreased during refrigerated storage ($p < 0.05$) and the decrease was more intense in the samples without chitosan. This effect may be explained by the fact that chitosan presents antioxidant properties which may contribute to maintain redness in muscle foods, due to its ability to act as a chelator on transition metal ions which catalyse oxidative reactions (*i.e.* oxidation of myoglobin).³¹ According to Georgantelis *et al.*⁷ chitosan could be chelating iron ions of meat hemoproteins during heat processing or storage. Similar results were also obtained for pork

Table 2 Color analysis during the storage period, for fresh goat sausages prepared with 2% chitosan and without chitosan (chitosan and control samples, respectively) and different amounts of fat 5% (F5), 12.5%(F12.5) and 20% (F20). Samples were stored at 4 °C for 15 days

Variables	Treatments (% of fat)	Storage period (days) ^a							
		0		5		10		15	
		Control	Chitosan	Control	Chitosan	Control	Chitosan	Control	Chitosan
<i>L^a</i>	F5	55.21 ± 1.13 ^{aD}	52.27 ± 1.46 ^{aE}	54.46 ± 0.65 ^{aC}	51.93 ± 0.88 ^{aD}	52.79 ± 0.97 ^{bB}	46.2 ± 1.99 ^{bC}	47.91 ± 1.02 ^{cC}	45.51 ± 0.82 ^{bD}
	F12.5	57.83 ± 0.76 ^{aAB}	55.52 ± 0.52 ^{aCD}	56.91 ± 0.24 ^{aBA}	54.96 ± 0.61 ^{aBC}	55.93 ± 0.43 ^{bA}	52.86 ± 1.10 ^{bb}	52.46 ± 1.15 ^{aB}	51.68 ± 0.86 ^{bB}
	F20	59.02 ± 1.03 ^{aA}	57.04 ± 0.43 ^{aBC}	58.07 ± 1.39 ^{aA}	56.33 ± 1.11 ^{aAB}	56.01 ± 1.91 ^{bA}	53.01 ± 1.60 ^{bb}	54.12 ± 0.77 ^{cA}	51.83 ± 0.83 ^{bb}
<i>a^a</i>	F5	8.51 ± 1.30 ^{aC}	12.49 ± 0.74 ^{aA}	4.48 ± 0.89 ^{bC}	10.39 ± 0.52 ^{bA}	2.48 ± 0.82 ^{cC}	9.54 ± 0.60 ^{bCA}	2.56 ± 0.20 ^{cA}	8.59 ± 0.36 ^{cA}
	F12.5	10.39 ± 0.29 ^{aB}	10.56 ± 0.22 ^{aB}	4.36 ± 0.51 ^{bC}	9.72 ± 0.63 ^{aAB}	2.82 ± 0.37 ^{cC}	8.08 ± 1.01 ^{bb}	3.33 ± 0.59 ^{bCC}	7.62 ± 0.68 ^{bAB}
	F20	7.60 ± 1.73 ^{aC}	10.14 ± 0.38 ^{aB}	3.81 ± 0.61 ^{bC}	9.03 ± 0.63 ^{aBB}	2.41 ± 0.39 ^{bCC}	8.03 ± 0.21 ^{bCB}	2.62 ± 0.28 ^{cC}	7.29 ± 1.20 ^{bB}
<i>b^a</i>	F5	12.09 ± 0.66 ^{aA}	10.51 ± 0.29 ^{aB}	10.87 ± 1.87 ^{bBC}	9.68 ± 0.50 ^{bb}	10.20 ± 0.82 ^{bb}	9.67 ± 0.49 ^{bb}	10.07 ± 0.55 ^{bb}	8.81 ± 0.40 ^{bC}
	F12.5	12.15 ± 0.65 ^{aA}	10.43 ± 0.81 ^{aB}	11.91 ± 0.28 ^{aAB}	10.50 ± 0.41 ^{aCD}	12.27 ± 0.45 ^{aA}	9.75 ± 0.39 ^{aB}	12.12 ± 0.68 ^{aA}	9.85 ± 0.41 ^{aBC}
	F20	12.90 ± 0.58 ^{aA}	10.71 ± 0.30 ^{aB}	12.30 ± 0.77 ^{aBA}	10.57 ± 0.75 ^{aBCD}	11.75 ± 0.97 ^{bA}	9.85 ± 0.64 ^{aBB}	11.31 ± 0.81 ^{bA}	9.65 ± 0.57 ^{bBC}

meat products with chitosan.^{12,18} Nitrate and nitrite are added to meat products due to their important role in color and flavor development and their antioxidant activity.⁹ However, *N*-nitrosamines (NA) may be formed increasing the demand for additive-free products by consumers. In this regard, we highlight the absence of chemical additives in our study, as well as, confirm the protective role of the chitosan showing a more stable and improved red color during refrigerated storage than the control samples.

Parameter b^* (yellowness) was not affected by the variation of fat level on day 0 and was always higher in the control samples than in samples added of chitosan ($p < 0.05$). This behavior may be due to the antioxidant properties of chitosan. However, generally the b^* values increase during storage with the intensity of the oxidation process that tends to increase the yellowness of samples caused by rancidification.³³ However, our results showed a slight difference in yellowness (b^*) throughout the storage time of fresh sausages, probably because the samples were stored for a short time and at refrigerated temperature.

Cooked samples analysis

Water retention capacity. The water retention capacity (Table 1) of the goat sausages after cooking was significantly affected by the variation of fat, time of storage and the addition of the chitosan ($p < 0.05$). Samples with higher fat levels (F20 and F20C) showed higher values of water retention capacity than the other samples with lower fat content (F5, F5C, F12.5 and F12.5C). According to Cavestany *et al.*³⁴ the higher the percentage of fat, the more concentrated and dense will be the emulsion's continuous phase, favoring, thus, the formation of the structure with greater water-holding ability. During storage, water retention capacity increases for both samples (with and without chitosan). This increase is probably due to the slight water loss during storage. Regarding the influence of chitosan, the control samples (F5, F12.5 and F20) showed a lower percentage of water retention capacity compared with the sausages with added chitosan (F5C, F12.5C and F20C), which also had the lowest moisture content. Claus *et al.*³⁵ reported that the lower moisture content provides a medium of greater ionic strength, which will lead to greater extraction of proteins and thus improves the binding properties. Similar behavior was reported by Sayas-Barberá *et al.*¹² in pork model burgers, which justified the highest cooking yield of samples with chitosan due to its water binding ability.

Texture profile analysis. Textural parameters are crucial to monitor the impact of chitosan and fat on the final sausage texture and consequently predict the impact on sensory quality. Among them hardness is one of the more relevant markers and represents the maximum force required to compress the sample. Results obtained from the fresh goat sausage texture (data not shown) showed that the addition of chitosan increased the hardness values (5.99 ± 0.07 to 7.27 ± 0.08) when compared with their control samples, without chitosan and the same level of fat (5.66 ± 0.25 to 5.81

± 0.49). The other textural parameters in general were not affected by the addition of chitosan. Regarding cooked goat sausage (Table 3), the sausages with chitosan showed higher hardness values than the control samples. The increase in hardness by adding of chitosan has been reported by García *et al.*³⁶ in pork sausage and Lin and Chao⁵ in reduced-fat Chinese-style sausage, and Amaral *et al.*¹⁸ in cooked pork sausage. This effect can be due to the fact that chitosan have the ability to act as a binder, thus favoring the formation of a stronger gel promoting a more stable structure.

After 15 days of refrigeration, the hardness of cooked goat sausage increased significantly ($p < 0.05$) in all groups, with this increase being significantly higher in the products with chitosan than in the control sample. This behavior can be explained not only by the slight drying of the product during storage (Ganhão *et al.*³⁷) but also due to the stabilization of chitosan linkages with matrix components at refrigerated temperature. Furthermore, this parameter showed a tendency to increase proportionally with the reduction of fat content. This result is consistent with those obtained by Cavestany *et al.*³⁴ that have assessed the effects of sardine surimi in Bologna sausage containing different fat levels, which reported that fat may act as a lubricant to allow myofilaments to slide past one another more easily, thus increasing tenderness and resulting in lower shear-force values. In our study, the lower hardness values were presented by the samples with higher fat content (F20 and F20C), which also showed the highest water retention capacity, as described before.

Regarding the other parameters of the texture profile, chitosan addition increased slightly the values of springiness, cohesiveness, gumminess, chewiness and resilience of cooked sausages compared with control sausages, though this tendency was not always significant. García *et al.*³⁶ in pork sausage reported that chitosan addition did not affect significantly the results of the texture profile analysis. Gumminess is the force necessary to disintegrate a semi-solid state sample until swallowing and chewiness is defined as the product of hardness and cohesiveness. These two parameters have their results dependent on the hardness, thus showing similar behavior, increasing with the addition of chitosan and time storage, as well as reducing with fat. The resilience was not affected by the addition of chitosan, reducing after 15 days of storage.

Principal component analysis (PCA) and Pearson correlation analysis

For a global view of the results presented in this work, a PCA (Fig. 2) and a Pearson correlation analysis (Table 4) were performed on microbial counts and physicochemical parameters, such as texture (by the TPA test) and color (L^* , a^* and b^*). On PCA, the resulting grouping of the variables analyzed in relation to the formulations (with and without chitosan; PC1) and storage time (PC2) showed 89% level of confidence. The microbiological parameters were all grouped in the right and lower part of the graph indicating the highest level of microorganisms for the sausages formulated without chitosan at 15

Table 3 Texture profile analysis obtained for cooking goat sausages prepared with 2% chitosan and without chitosan (chitosan and control samples, respectively) and different amounts of fat 5% (F5), 12.5% (F12.5) and 20% (F20). Samples were stored at 4 °C for 15 days

Variables	Treatments (% of fat)	Storage period (days) ^a					
		0		5		10	
		Control	Chitosan	Control	Chitosan	Control	Chitosan
Hardness (N)	F5	13.56 ± 1.60 ^{aAB}	14.01 ± 0.82 ^{bAB}	14.04 ± 0.60 ^{aAB}	16.20 ± 1.02 ^{bAB}	14.33 ± 1.31 ^{AB}	17.76 ± 0.63 ^{BA}
	F12.5	11.71 ± 3.36 ^{abABC}	14.57 ± 0.68 ^{BA}	11.77 ± 1.55 ^{abB}	14.31 ± 3.87 ^{bAB}	10.65 ± 1.00 ^{BC}	15.62 ± 0.63 ^{abAB}
	F20	9.54 ± 1.24 ^{AC}	10.91 ± 3.21 ^{bBC}	11.42 ± 1.57 ^{abB}	13.29 ± 1.44 ^{abAB}	9.18 ± 1.28 ^{AC}	13.9 ± 0.90 ^{AB}
Springiness (mm)	F5	0.93 ± 0.02 ^{BA}	0.95 ± 0.03 ^{BA}	0.92 ± 0.04 ^{AB}	0.95 ± 0.06 ^{BB}	0.89 ± 0.02 ^{BA}	0.98 ± 0.03 ^{BA}
	F12.5	0.96 ± 0.05 ^{BA}	0.96 ± 0.08 ^{BA}	0.87 ± 0.02 ^{AB}	1.32 ± 0.43 ^{BA}	0.94 ± 0.00 ^{AA}	0.98 ± 0.04 ^{BA}
	F20	0.90 ± 0.05 ^{BA}	0.95 ± 0.04 ^{BA}	0.91 ± 0.07 ^{AB}	0.95 ± 0.04 ^{AB}	0.88 ± 0.02 ^{BA}	0.96 ± 0.05 ^{BA}
Cohesiveness	F5	0.33 ± 0.11 ^{BB}	0.38 ± 0.06 ^{AB}	0.41 ± 0.04 ^{AB}	0.43 ± 0.09 ^{AB}	0.45 ± 0.12 ^{ABC}	0.40 ± 0.02 ^{ABC}
	F12.5	0.37 ± 0.07 ^{BB}	0.33 ± 0.05 ^{BB}	0.50 ± 0.11 ^{AB}	0.56 ± 0.00 ^{AA}	0.42 ± 0.02 ^{ABC}	0.47 ± 0.07 ^{ABC}
	F20	0.50 ± 0.04 ^{ABA}	0.43 ± 0.02 ^{BAB}	0.41 ± 0.07 ^{BB}	0.43 ± 0.05 ^{BB}	0.55 ± 0.01 ^{AB}	0.57 ± 0.03 ^{AB}
Gumminess (N)	F5	5.10 ± 0.84 ^{AA}	5.22 ± 0.80 ^{CA}	5.78 ± 0.68 ^{AB}	6.41 ± 1.53 ^{bCA}	6.57 ± 1.86 ^{abABC}	7.16 ± 0.04 ^{abAB}
	F12.5	4.21 ± 0.94 ^{AA}	4.85 ± 0.92 ^{CA}	5.74 ± 0.53 ^{AB}	5.76 ± 1.03 ^{bCA}	5.20 ± 0.64 ^{ABC}	7.32 ± 0.90 ^{ABA}
	F20	5.40 ± 1.21 ^{AA}	5.86 ± 1.70 ^{abAB}	5.10 ± 0.43 ^{AB}	4.23 ± 1.23 ^{BB}	4.95 ± 0.33 ^{AC}	6.35 ± 1.35 ^{ABC}
Chewiness (N mm)	F5	4.73 ± 0.87 ^{AB}	5.27 ± 0.43 ^{BAB}	5.33 ± 0.81 ^{AB}	6.73 ± 1.40 ^{BA}	5.21 ± 2.05 ^{abABC}	7.02 ± 0.21 ^{ABA}
	F12.5	3.66 ± 0.49 ^{BB}	4.61 ± 0.64 ^{BAB}	4.68 ± 1.07 ^{abAB}	6.52 ± 0.97 ^{abAB}	3.82 ± 0.73 ^{ABC}	7.14 ± 0.59 ^{BA}
	F20	4.13 ± 0.20 ^{AB}	5.77 ± 1.39 ^{ABA}	4.64 ± 0.51 ^{AB}	4.46 ± 0.38 ^{BB}	4.65 ± 0.74 ^{ABC}	6.56 ± 1.65 ^{AB}
Resilience	F5	0.11 ± 0.08 ^{abB}	0.10 ± 0.04 ^{AB}	0.11 ± 0.05 ^{abAB}	0.10 ± 0.01 ^{AB}	0.16 ± 0.08 ^{AB}	0.10 ± 0.03 ^{ABC}
	F12.5	0.11 ± 0.05 ^{abB}	0.10 ± 0.04 ^{AB}	0.18 ± 0.07 ^{AA}	0.19 ± 0.04 ^{AB}	0.21 ± 0.00 ^{BC}	0.09 ± 0.04 ^{ABC}
	F20	0.23 ± 0.07 ^{AA}	0.25 ± 0.03 ^{BA}	0.10 ± 0.06 ^{AB}	0.14 ± 0.05 ^{BAB}	0.21 ± 0.03 ^{ABA}	0.05 ± 0.10 ^{BABC}

^a Different letters (a, b) in the same row differ significantly ($p < 0.05$) in time. Different letters (A, B) in the same column differ significantly ($p < 0.05$) in samples.

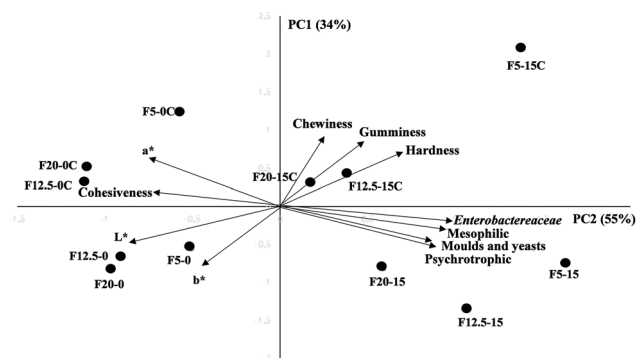


Fig. 2 Principal component analysis (PCA) for the textural parameter (cooked sample), color and microbiological analysis (fresh sample) of goat sausages prepared with different amounts of fat, 5%, 12.5% and 20% and with 2% chitosan (samples F5C, F12.5C and F20C) and without chitosan (samples F5, F12.5 and F20) on 0 and 15 days of storage at 4 °C. A confidence interval of 95% ($p < 0.05$) was considered in all cases.

days (F5-15, F12.5-15 and F20-15). Since, for all the groups of microorganisms analyzed the increase in storage time led to increase of microbial levels, the significant correlation was expected. Therefore, the data was not shown in the Pearson correlation table. The texture parameters (hardness, gumminess and chewiness) were all grouped in the right and upper part of the graph, showing the highest values in all the sausages with chitosan after 15 days of storage (F5C-15, F12.5C-15 and F20C-15). This was due to the chitosan incorporation and the time of storage that increased the compressive forces and, therefore, resulted in an improvement of the texture. The correlation between hardness, gumminess and chewiness is explained because these two last parameters have their results dependent on the hardness, as previously mentioned. Finally, the color parameters showed a trend of reduction with storage time. The L^* and b^* showed a correlation and were grouped in the left and lower part of the graph, possibly because they have similar behavior, such as, a decrease over time and with the addition of chitosan. Already the a^* located on the left and upper part of the graph was affected by chitosan, thus improving the red color of goat sausage, resulting in positive effects in appearance.

Experimental

Sausage ingredients

Sausages were formulated according to previous studies.¹⁸ Thus, ingredients such as salt, fresh garlic, powder white pepper and dried oregano were obtained in local markets of the city of Porto (Portugal). Goat meat was removed from the leg, shoulder, rib, neck and loin cuts of male animals, without defined breed, slaughtered between 8 and 10 months old and artificial casings were also bought in traditional local markets.

Chitosan was provided by Sigma-Aldrich (Steinheim, Germany) and previously characterized in the laboratory.³⁸

Table 4 Pearson correlation between textural parameter (cooked sample) and color analysis (fresh sample) of goat sausages prepared with different amounts of fat. 5% (F5), 12.5% (F12.5) and 20% (F20) and with 2% chitosan and without chitosan stored at 4 °C for 15 days

	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness	<i>L</i> *	<i>a</i> *	<i>b</i> *
Hardness		−0.241 ^{ns}	−0.443 ^{ns}	0.894**	0.791*	−0.898**	−0.025 ^{ns}	−0.766*
Springiness			0.710 ^{ns}	−0.071 ^{ns}	0.188 ^{ns}	0.482 ^{ns}	0.403 ^{ns}	0.085 ^{ns}
Cohesiveness				−0.079 ^{ns}	0.100 ^{ns}	0.608*	0.471 ^{ns}	0.097 ^{ns}
Gumminess					0.902**	−0.764*	0.141 ^{ns}	−0.823*
Chewiness						−0.572 ^{ns}	0.285 ^{ns}	−0.663*
<i>L</i> *							0.315 ^{ns}	0.774*
<i>a</i> *								−0.090 ^{ns}
<i>b</i> *								

ns – not significant. *Significant at $p < 0.05\%$ probability. **Significant at $p < 0.01\%$ probability.

Sausage manufacture

Fresh sausage manufacture and chitosan addition were carried out under the same procedure as indicated in a previous study.¹⁸ In brief, three different formulations were made with 2% (w/w) of chitosan and different fat concentrations: 5% (w/w) (Formulation F5C), 12.5% (w/w) (Formulation F12.5C) and 20% (w/w) (Formulation F20C). A sample without chitosan was used as a control for each formulation (F5, F12.5 and F20). Each fresh sausage weighed 50 g and had 3 cm of diameter. They were packed in plastic bags without vacuum and stored under refrigeration at 4 °C for 15 days. One lot of 1000 g of fresh goat sausage of each formulation was prepared and divided into two replicates for consequent analysis.

Microbiological analysis

Mesophilic and psychrophilic bacteria, *Enterobacteriaceae* and yeast and molds were determined over the storage period at 4 °C in the samples on days 0, 5, 10 and 15. For this, 8 g of sample were placed in plastic bags and homogenized for 2 min in a stomacher (Lab Blender 400, London, UK) with 80 ml of sterile 0.1% peptone water. The homogenate was serially diluted using the peptone water as a diluent and plated in duplicate using the drop method (20 µl of each dilution) to enumerate viable counts of mesophilic and psychrophilic bacteria (PCA-Plate Count Agar, Biokar diagnostics) incubated at 30 °C for 48 h and 7 °C for 7 d, respectively and yeasts and molds (PDA-Potato Dextrose Agar, Biokar diagnostics) incubated at 25 °C for 5 d. *Enterobacteriaceae* (VRBGA-Violet Red Bile Glucose Agar, Lab) were evaluated by the pour plate technique³⁹ incubated at 37 °C for 24 h. After incubation, the colonies were enumerated and colony forming units (cfu mL^{−1}) were calculated. Each replicated sample of fresh goat sausages was analysed in duplicate.

Physicochemical analyses

Fresh samples

Proximate analysis, lipid oxidation and color analysis. Proximate analysis was determined in the samples at 0, 5, 10 and 15 days of storage at 4 °C. Moisture, ash, protein, and fat content were determined by the official AOAC methods of analysis 24.003, 24.009, 24.027, and 24.005, respectively.⁴⁰ Results were expressed in all cases as g per 100 g of sample.

The pH values of the samples were also measured by an AOAC method of analysis. Specifically, it was analyzed by the 943.02 method.⁴¹ A combined pH glass electrode connected to a pH-meter MicroPH 2001 Crison potentiometer (MicroPH 2001, Barcelona, Spain) was used. Each replicated sample of fresh goat sausages was analysed in duplicate.

Lipid oxidation

Lipid oxidation was assessed by measuring the thiobarbituric acid reactive substances (TBARS). 2 g of sample were homogenized by vortexing in 10 mL of 10% of trichloroacetic acid (TCA biochemical/Applichem) and 5 ml of 0.02 M 2-thiobarbituric acid (TBA, Merck). Then, it was centrifuged at 5000 rpm for 20 min in a UNiversal 320R centrifuge (Zentrifugem, HETTICH). The supernatant was collected and filtered, heated in boiling water for 35 min at 100 °C and chilled in iced water for 10 min. Finally, absorbance at 532 nm was measured in a spectrophotometer UV mini 1240 (Shimadzu, Tokyo, Japan). 1,1,3,3-Tetraethoxypropane (Sigma Aldrich) was used as a standard in the range 1×10^{-6} – 14×10^{-6} mol L^{−1}. TBARS concentration was expressed as mg malondialdehyde per kg of sample. Each replicated sample of fresh goat sausages was analysed in duplicate.

Color analysis

Color was determined as previously reported,¹⁸ using a digital Minolta colorimeter (Model CR-300, Minolta, Osaka, Japan). The parameters lightness (*L**), redness/greenness (*a**) and yellowness/blueness (*b**) were determined according to the specifications of the Commission Internationale de L'éclairage (CIE, 1986), being, illuminant D65, 8° viewing angle, standard observer angle of 10° specular included. The determinations in each replicated sample of fresh goat sausages were performed in triplicate.

Cooked samples

Two fresh goat sausages of each formulation were subjected to cooking after 0, 5, 10 and 15 days of storage at 4 °C. For this, the sausages were cooked in hot water until reaching 72 °C at the geometric center, controlled by a thermocouple, then were taken and maintained at room temperature until cooling. Since chitosan impacts especially on moisture and fat reten-

tion, as well as some textural parameters, those parameters were also analyzed in cooked samples.

Water retention after cooking

The water holding capacity in cooked samples was determined according to the methodology previously described by other authors.¹² The estimated amount of moisture retained in the samples was calculated according the following equation:

$$\begin{aligned} \% \text{ Water retention} &= 100 \times \text{cooked weight (g)} \\ &\times \% \text{ moisture in cooked sample raw weight (g)} \\ &\times \% \text{ moisture in raw sample} \end{aligned}$$

Texture profile analysis

Fresh and cooked samples were subjected to textural analysis. Thus, two sausages of each formulation at 0, 5, 10 and 15 days of storage at 4 °C (fresh and cooked) were cut into three pieces of 3 cm high and compressed twice using a texture analyzer TA-XT2 (Stable Micro Systems, Haslemere, England). Textural parameters were measured by compressing with a cylinder probe of 2 cm in diameter. Force–time curves were recorded at a crosshead speed of 5 mm s⁻¹ at a distance of 35 mm. Hardness (peak force of first compression cycle, N), chewiness (hardness × cohesiveness × springiness, N × mm), cohesiveness (ratio of positive areas of second cycle to area of first cycle, dimensionless), gumminess (hardness × cohesiveness, N), springiness (distance of the detected height of the product on the second compression divided by the original compression distance, mm mm⁻¹) and resilience (area during the withdrawal of the first compression divided by the area of the first compression) were the textural parameters.

Statistical analysis

The statistical package used was SAS version 9.4 (2013) to explore the statistical significance of results. The analysis of variance (ANOVA) using the Tukey test was applied to the results of physicochemical and microbiological analysis to determine the statistically significant differences between formulations during storage. Data from microbiological, texture and color analysis were evaluated by principal component analysis (PCA) and Pearson correlation analysis. A confidence interval of 95% ($p < 0.05$) was considered in all cases.

Conclusions

The use of chitosan in the manufacture of fresh goat sausages has been studied for the first time. A better preservation of quality and extension of the product shelf life was observed through a significant reduction of microbial growth and lipid oxidation in chitosan added sausages, when compared with the controls. Moreover, an enhancement of the physical stability of sausages with chitosan was also obtained with an improvement in the red color and also in a firmer texture through the increase of hardness. Although it is necessary to conduct further studies to show the beneficial properties of

this product, the results included in the present study indicate that incorporation of 2% (w/w) chitosan in goat meat sausage is technologically feasible to formulate a product with a reduced fat content and, at the same time, meet the requirements of the EFSA (ingestion 3 g chitosan per day; 3 sausages) to cause a decrease in serum cholesterol.

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ARTICLE 4**EFFECT OF CHITOSAN AND CHITOSAN–GLUCOSE MAILLARD
REACTION PRODUCT ON QUALITY AND SHELF LIFE OF LOW FAT
GOAT MEAT SAUSAGE**

**LOW FAT GOAT MEAT SAUSAGE WITH CHITOSAN–GLUCOSE MAILLARD
REACTION PRODUCT: IMPACT ON QUALITY AND SHELF LIFE**

Deborah S. do Amaral^a, Alejandra Cardelle-Cobas^b, Samara de A. Silva^a, Celina de Castro Q. Dias^a, Naciza Maria de O. Arcanjo^a, Paulo S. Dalmás^c, Marta S. Madruga^a, Maria Manuela E. Pintado^{*b}

^aDEA – Departamento de Engenharia de Alimentos, Centro de Tecnologia, Universidade Federal da Paraíba, 58051-900 João Pessoa, Paraíba, Brasil

^bCBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal

^cIFSERTÃO - Centro de Tecnologia de Alimentos, Instituto Federal do Sertão Pernambucano, BR 407, s/n - Jardim São Paulo, 56314-520, Petrolina – Pernambuco, Brasil.

*Corresponding author:

E-mail address: mpintado@porto.ucp.pt

Tel.: +351-225580097

Fax: +351-225090351

Abstract

Low fat fresh goat sausages based on chitosan (LMWC) and derivative chitosan (LMWC-Glc) were developed aiming to produce a functional food product evaluating the effect of inclusion of these compounds on quality, stability and shelf life. Goat sausages (10% pork fat) were formulated with 2% (w/w) of LMWC or LMWC-Glc and stored at 4 °C during 21 days. Results indicated that incorporation of LMWC-Glc and LMWC is technologically feasible, originating a potential functional product to claim reduction of cholesterol, besides the positive impact on microbial safety and lipid oxidation. The modified LMWC (towards better solubility and lower adstringency) incorporated at 2% as functional ingredient showed lower antioxidant activity than the native chitosan, but maintained positive effect on antimicrobial, nutritional, textural and sensory performance. The results also proved that derivative chitosan may also be a promising strategy for improving the quality and extending the shelf life of low fat goat sausage.

Keywords: chitosan, chitosan–glucose, functional meat product, fat reduction, Maillard reaction, cholesterol reduction

1. Introduction

Goats are ruminants that stand out by a good adaptability, characteristic that allows its breeding on pastures not easily accessible to other livestock species (Palo et al., 2016). In Brazil, the northeast region has 91% of the national goat population (Lopes et al., 2012), being an economically important activity (Nassu et al., 2003) besides its relevant nutritional value either for the milk or the meat. The goat is known to produce relatively lean meat (Banskalieva et al., 2000), combined with its high digestibility, high protein content, and richness in iron and unsaturated fatty acids, when compared to other red meats (Madruga, 2004). Hence, the best cuts of goat meat are well valorized and sold at high prices, however the remaining cuts although still with high nutritional value have low consumer acceptability and no commercial value (Nassu et al., 2003). Therefore, the use of meat from low value goat cuts to produce fresh goat sausages may be an interesting way to valorize the product with very low market acceptability, satisfying the interest of producers and introducing in the market a new meat product (Leite et al., 2015).

Sausages are one of the most popular food product consumed worldwide (Purohit et al., 2016), which traditionally consists of chopped meat, water, binders, fat, and seasonings (Essien, 2003). However, as processed meat product, they have short shelf-life due to high probability of microbial cross- and post-contamination, as well as by the facilitated oxidation of exposed meat lipids (in particular unsaturated fatty acids), requiring preserving additives to assure safe and good quality products. So, the addition of phosphates, nitrates and nitrites is permitted (Georgantelis et al., 2007). However, the utilization of nitrite has been limited by the risk of N-nitrosamines formation, a group of compounds that are well known for their carcinogenic and mutagenic activities (Wang et al., 2015).

In response to recent claims that synthetic antioxidants have the potential to cause toxicological effects and the consumers' increased interest in purchasing natural products, the meat industry has been seeking sources of natural antioxidants (El-Nashi et al., 2015). Among these different natural compounds, chitosan, usually obtained from crustaceans byproducts (present in the exoskeletons), has attracted especial interest from the meat industry as a potential natural food preservative due to possess important functional activities such as antimicrobial, antioxidant, among others (Shahidi et al., 1999), besides be non-toxic, biodegradable and biocompatible (Harish Prashanth & Tharanathan, 2007).

Previous studies indicated that chitosan possess good potential to increase the stability and shelf-life of pork sausage (Amaral et al., 2015; García et al., 2010; García et al., 2011; Soutos et al.; 2008; Georgantelis et al., 2007), goat sausage (Amaral et al., 2016), bovine pâté (Bento et al., 2011), pork burgers (Sayas-Barberá et al., 2011), salami (Kanatt et al., 2008) and cured chicken meat (Shekarforoush et al., 2015) among others meat products. However, despite the positive effects it is possible to find in the literature many references on the perception of astringency (Amaral et al., 2015) and little detrimental effects on the textural properties (Chang et al., 2011) in food matrices incorporated with chitosan, besides its low solubility in water (Ruiz Matute et al., 2013). Thus, in order to circumvent these problems it is desirable to modify chitosan, but without affecting its antimicrobial and antioxidant activity (Jiang et al., 2012).

In this context, several researchers have developed methods to improve the properties of chitosan using chemical and enzymatic modifications, although the chemical modifications are generally not preferred in food applications (Rao et al., 2011), due to the formation of potential side detrimental products (Kanatt et al., 2008). The presence of amino groups makes chitosan prone to react with the carbonyl group of a reducing sugar (e.g. glucose), and allows it to be a participant in the Maillard reaction (Chang et al. 2011; Jiang et al.,

2012). This reaction has been indicated as a promising and facile method for commercial manufacture of derivative chitosans (Chung et al., 2005; 2006; Gullón et al. 2015; Ying et al., 2011).

In assessing the synthesis, optimization and structural characterization of a chitosan-glucose derivative obtained by the Maillard reaction Gullón et al. (2015) confirmed the successful introduction of glucose into the chitosan molecule and indicated that the derivative of chitosan can be used in the food industry. Chang et al. (2011) have studied the effect of chitosan–glucose Maillard reaction product on pork qualities during refrigerated storage, reporting lower TBARS values and microbial counts, without negative influences on the sensory qualities of the samples. Similarly, Kanatt et al. (2008) in raw lamb meat reported that the use of chitosan and chitosan-glucose complex increased by more 2 weeks the shelf life of the treated samples compared to controls. They concluded that both these compounds can be used in meat formulations as preservatives. Regarding the functional benefits Amaral et al. (2015) indicated that incorporation of 2% chitosan into pork sausages to ensure the EFSA claim of reduction of cholesterol is technologically feasible and also allows to obtain a product with improved properties, namely if fat reduction is sought. However, the application of chitosan-glucose derivative obtained by the Maillard reaction in processed goat meat products or in a range of concentrations enough to reach the health claim was not yet established. Therefore, the objective of this study was to include chitosan (control) and chitosan–glucose complex in a fresh low fat goat sausage in an adequate concentration to be in accordance with EFSA recommendation to support a decrease in serum cholesterol (3g chitosan/day) and establish the effect on the quality, stability and shelf life of the product stored at 4 °C during 21 days through the evaluation of the microbiological, physico-chemical and sensory parameters.

2. Materials and methods

2.1. Sausages ingredients and chitosan

The goat meat of male animals, not defined breed, slaughtered between 8 and 12 months old, pork fat, ingredients (salt, garlic, onion, powder black pepper, dried oregano, starch and soy protein isolate) and artificial casings were obtained in local markets in the city of Petrolina (Brasil).

Chitosan used in the study was Low Molecular Weight Chitosan (LMWC, Sigma-Aldrich, Steinheim, Germany), previously characterized (Gullón et al. 2015), with a Molecular Weight (MW) of 123 KDa and 90% deacetylated. The derivative of chitosan (chitosan–glucose Maillard reaction product) obtained by the introduction of glucose was produced using the methodology described in Gullón et al. (2015). Characterization of the derivative chitosan (LMWC-Glc) indicated a MW of 210.37 kDa and a degree of substitution of Glc of 65%, being 25%, the percentage of free amino groups.

2.2. Sausage preparation

Three lots of low fat sausage (10% pork fat) were manufactured varying the type of chitosan, such as LMWC, LMWC-Glc and a control formulation without chitosan. The base formulation of the sausage previously tested as optimal was prepared using 75% (w/w) of minced goat meat, 10% (v/w) of water, 10% (w/w) fat, 1.5% (w/w) of salt, 0.1% (w/w) of powder garlic, 0.2% (w/w) of powder onion, 0.1% (w/w) of black pepper, 0.05% (w/w) of dried oregano, 2% (w/w) of starch and 2% (w/w) of soy protein isolate. LMWC and LMWC-Glc were added to 2% (w/w), being this amount sufficient to accomplish the EFSA

specifications for hypocholesterolemic effects of chitosan, assuming the ingestion of 3 g chitosan/day corresponding to 3 sausages of ca. 50g/each.

For the preparation of sausages the minced goat meat was mixed with salt, fat, water and other ingredients (garlic and onion powder, black pepper, starch, oregano, isolated soy protein and chitosan or derivative) in cutter table (JAMAR, model K-10, São Paulo, Brazil). In the case of the derivative LMWC-Glc owing to its soluble character, this was moistened with part of the water to then be added to the formulation assuring better homogenization. After homogenization, the mixture was embedded into artificial casings, using a manual stuffer type cannon (SIEMSEN LTDA, CFMN model ES-08, Brusque, SC, Brazil) with capacity for 3 kg, obtaining fresh goat sausages with 3 cm of diameter and 50 g per unit. The sausages were then placed in plastic bags without vacuum and stored at 4 °C for up to 21 days. One lot of 1000 g of fresh goat sausage of each formulation was prepared and two replicates of each sample were analyzed in triplicate.

2.3. Microbiological analysis

The microbiological quality of fresh goat sausages was assessed on days 0, 7, 14 and 21 of storage at 4 °C. Thus, 8 g of sample in 80 mL of sterile peptone water were placed in plastic bags and homogenized for 2 min in a stomacher (Lab Blender 400, London, UK). The homogenate was serially diluted in the same diluent and plated in duplicate using the drop method (20 µl of each dilution), as described by Miles et al. (1938) to evaluate the viable counts of mesophilic and psychrophilic bacteria (plate count agar - PCA, Biokar diagnostics) incubated at 30 °C for 48 h and 7 °C for 7 days, respectively, as well as yeasts and molds (Potato Dextrose Agar - PDA, Biokar diagnostics) being incubated at 25 °C for 5 days. Enterobacteriaceae were enumerated by the pour-overlay method using Violet Red Bile

Glucose Agar (VRBGA, Lab) and the corresponding plates incubated at 37 °C for 24 h (ISO, 2004). After incubation, the colonies were enumerated and the colony forming units (CFU/mL) were calculated.

2.4. Physicochemical analyses

2.4.1. Proximate composition and pH values

Moisture, ash, protein, and fat content of samples were determined, in raw sausages, at 0, 7, 14 and 21 days of storage at 4 °C by the official AOAC methods of analysis 24.003, 24.009, 24.027, and 24.005, respectively (AOAC, 1990). In brief, the methodology is described as follows: Moisture (g water/100 g sample) was determined by drying the samples at 105 °C to constant weight. Ash content (g ash/100 g sample) was calculated after incineration of the samples in a muffle at 550 °C and weighed. Protein (expressed as g protein/100 g sample) was analyzed by the Kjeldahl method. Fat content (g fat/100 g sample) was calculated by cold extraction with chloroform/methanol (2:1) by the method of Folch et al. (1957).

The pH values of samples were also measured by a AOAC method of analysis. Specifically they were analyzed by the 943.02 method (AOAC, 2000). A combined pH glass electrode connected to a pH-meter MicropH 2001 Crison potentiometer (MicropH 2001, Barcelona, Spain) was used.

2.4.2. TBARS determination

Lipid oxidation was assessed by measuring the thiobarbituric acid reactive substances (TBARS) by the method adapted from Rosmini et al. (1996). Thus, 2 g of sample were

homogenized by vortexing in 10 mL of 10% (v/v) of trichloroacetic acid (TCA biochemical/Applichem) and 5 ml of 0.02 M 2-thiobarbituric acid (TBA, Merck). Then, it was centrifuged at 5000 rpm for 20 min in a Universal 320R centrifuge (Zentrifugem, HETTICH). The supernatant was collected and filtered, heated in boiling water for 35 min at 100 °C and chilled in iced water for 10 min. Finally, absorbance at 532 nm was measured in a spectrophotometer UV mini 1240 (Shimadzu, Tokyo, Japan). 1,1,3,3 tetraethoxypropane (Sigma Aldrich) was used as standard in the range 1×10^{-6} – 14×10^{-6} mol/L. TBARS concentration was expressed as mg malondialdehyde per kg of sample.

2.4.3. Instrumental color analysis

Color measurements of the fresh goat sausage was determined according to methodology described by Abularach, Rocha, and Felício (1998) using a digital Minolta colorimeter (Model CR-300, Minolta, Osaka, Japan). To read the L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) parameters, the following conditions were set: illuminant D65, a viewing angle of 88, a standard observer's angle of 108, with the specular component included according to specifications of the Commission Internationale de L'eclairage (CIE, 1986).

2.4.4. Fatty acid profile determination

Fatty acids were transesterified following the method described by O'Fallon et al. (2007) with some modifications. Thus, 1 g of sample were homogenized in test tube with 1 ml of heneicosanoic acid (C21:0 in 1mg / ml) used as internal standard, 5.3 ml methanol and 0.7 ml of 10 N KOH solution vortexed for 10 s and placed on heated in water at 55 °C for 1.5

h, after were cooled in cold water, add 0.58 mL of 24N H₂SO₄ solution, vortexed and brought back to heating in water at 55 °C with stirring for 1.5 h. Then, cooled in cold water, add 3 ml of hexane and vortexing for 3 min. Two cycles of centrifugation were carried out for 5 min each to extract the hexane phase and used 0.5 g of anhydrous sodium sulfate as drying agent. After centrifugation, 1.5 ml of hexane phase was put in vial. The separation and quantification of fatty acid esters was performed by gas chromatography (HP Hewlett Packard 6890 Series GC System) coupled with a FID detector and a fused silica capillary column (CP WAX 52 CB, VARIAN) with film thickness dimensions of 100 m x 0.25 mm and 0.25 µm. Helium was used as the carrier gas (1 ml/min flow rate). The initial oven temperature was 40 °C/ 2 min programmed to increasing 10 °C/min reach 180 °C for 30 minutes and 10 °C/min reach 240 °C for 34 min. The injector and detector temperatures were maintained at 250 °C and 260 °C, respectively. Fatty acid esters were identified using software EZChrom Elite, version 3.3.2 SP2 Agilent, EUA, 2005- 2010 and expressed as mg/g of fatty acids.

2.4.5. Determination of cholesterol content

The method of Folch et al. (1957) was used to extract the lipids of goat sausages and the cholesterol content was measured according to methodology proposed by Bragagnolo and Rodriguez-Amaya (1997). A high resolution liquid chromatography instrument (VARIAN, Waters 2690, California, USA) with an INESTISIL C18 column (4.6 mm x 150 mm x 5 mm) was employed for the determination of the cholesterol content in the samples. An isocratic elution of acetonitrile and isopropanol (60:40) with a constant flow rate of 1 mL/min was used as the mobile phase. The detection of total cholesterol occurred in a UV-VIS detector

(PDA 330) set at 210 nm. The chromatographic separation was performed at 30 °C with a run time of 10 min.

2.4.6. Moisture and fat retention

The moisture content were determined. Two sausage of each formulation were cooked to a core temperature of 72 °C. Internal temperature was determined at the geometric center of the sausages by inserting thermocouples. After cooking, sausages were maintained at room temperature until cooling and were weighed and measured before and after cooking. The estimation the amount of moisture retained in the samples was determined according the methodology describe by Sayas-Barberá et al. (2011) using the following equation:

$$\% \text{ Moisture retention} = 100 \times \frac{\text{cooked weight (g)} \times \% \text{ moisture in cooked sample}}{\text{raw weight (g)} \times \% \text{ moisture in raw sample}}$$

2.4.5. Texture profile analysis (TPA)

Two fresh sausages of each formulation were subjected to cooking to reach 72 °C at the geometric center after the days 0, 7, 14 and 21 of storage at 4 °C, then were maintained at room temperature until cooling. Four pieces of 1 cm of each sausage were compressed twice using a texture analyzer (Model TA.XT Plus, Stable Micro Systems, Surrey, England). A cylindrical probe of 6 mm diameter adaptor with 2 mm/ s test speed and 70% compression was used to determine the parameters of hardness, springiness, cohesiveness, gumminess, chewiness and resilience (Lin and Chao, 2001). Hardness (N) is the maximum force required to compress the sample; cohesiveness is the extent to which sample could be deformed prior to rupture (A_2/A_1), being A_1 the total energy required for the first compression and A_2 the

total energy required for the second compression; springiness (mm) is the ability of the sample to recover its original shape after the deforming force is removed; gumminess (N) is the force to disintegrate a semisolid meat sample for swallowing (hardness $\times$ cohesiveness), chewiness (N $\times$ mm) is the work needed to masticate the sample for swallowing (springiness $\times$ gumminess) and resilience (area during the withdrawal of the first compression divided by the area of the first compression) (Bourne, 1978).

2.5. Sensory Analysis

The quantitative descriptive analysis (QDA) was used to evaluate the sensory quality of the goat sausage, using a trained panel of nine members. The preparatory sessions were held before the test, so that each panel member could discuss and clarify each attribute being evaluated (Soltos et al., 2008). The sensory test was initiated after the panellists agreed on the specifications. The sausages were grilled in an electrical grill to a core temperature of 72 °C, which was monitored at the geometric center of the sausages using a thermocouple (Soulto et al., 2008). After cooling for 3 min at room temperature, the samples were cut into pieces of 3 cm and served to each panelist separately, together with water and biscuit cream cracker to cleanse the palate. Attributes of taste, appearance, flavour and texture were evaluated at days 0 and 7 of storage and at day 14 only the appearance and flavor. The sensory evaluation was conducted using a structured scale of 9 cm, with 1 representing the lowest intensity and 9 the highest intensity, for all attributes.

2.6. Statistical analysis

Data were analyzed using the statistical package SAS version 9.4 (2013). The analysis of variance (ANOVA) using the Tukey test was applied to the results of physicochemical, microbiological and sensory analysis to determine the statistically significant differences between formulations during storage. The statistical significance was estimated at 5% level ($p < 0.05$) in all cases.

3. Results and discussion

3.1. Microbiological analysis

The results of microbiological analyses of the fresh goat sausages throughout the 21 days storage period are presented in Table 1. The counts of all determined microbiological indicators were significantly affected ($p < 0.05$) by the addition of chitosan and derivative chitosan, as well as, by storage time. In general, the samples treated (with LMWC and LMWC-Glc) had lower microbial counts compared to control sample. Furthermore, it is possible to observe that microbiological counts increased, in all cases as expected, throughout the storage time. In the beginning (day 0) mesophilic bacteria showed levels of 6.10 ± 0.05 and 6.03 ± 0.10 log CFU/g for the sausages with LMWC and LMWC-Glc, respectively, with no statistically difference ($p > 0.05$), but higher and significantly different (6.28 ± 0.03) for the control sausages. Similar behaviour was found in the counts of moulds and yeasts and *Enterobacteriaceae*, which showed lower values for the sausages with LMWC and LMWC-Glc than in control sausages. In regarding the psychrophilic bacteria its growth was only reduced by the presence of LMWC (4.87 ± 0.10), while LMWC-Glc

(5.36 ± 0.05) had no inhibitory effect, showing slightly higher scores than the control sample (5.15 ± 0.08), at 0 day.

After 21 day of storage at 4° C the mesophilic bacteria counts were approximately 0.8 log CFU/g lower in the samples treated with 2% of LMWC and LMWC-Glc, 8.46 ± 0.04 and 8.41 ± 0.05 , respectively, than the control samples, 9.21 ± 0.08 , ($p < 0.05$). Counts of moulds and yeasts, psychrophilic and *Enterobacteriaceae* also increased significantly for all samples ($p < 0.05$) after 21 days at 4 °C, remaining always lower in samples added LMWC and LMWC-Glc, which promoted a reduction of counts about 0.5 log CFU/g compared to the control sausage.

These result is consistent with those reported by other researchers, who demonstrated that the addition of chitosan resulted in antimicrobial effect (Amaral et al. 2015; Georgantelis et al., 2007; Roller et al., 2002; Sayas-Barberá et al., 2011; Soltos et al., 2008). Soultos et al (2008) in fresh pork sausage after 21 days of storage, reported a similar inhibiting effect to our by adding of 1% chitosan (0.5 log CFU/g) for the total viable counts, moulds and yeasts and *Enterobacteriaceae*, as well as they reported a slightly higher reduction to our (0.9 log CFU/g) for counts of *Pseudomonas* spp. compared to the control sausage. Amaral et al. (2015) in fresh pork sausages prepared with different levels of fat (5, 12.5 and 20%) reported that chitosan incorporation (2% w/w) induced, in general, a significant reduction of viable cells (ca. 0.5–1.0 log CFU mL⁻¹) of mesophilic, psychrophilic, *Enterobacteriaceae* and moulds and yeasts throughout the storage time. This inhibitory effect has also been observed for the chitosan-glucose complex. Jiang et al. (2012) reported a reduction of counts of 1.5 log CFU/g of mesophilic bacteria and moulds and yeasts and 2 log CFU/g of *Pseudomonas* counts in shiitake mushrooms treated with chitosan–glucose complex coating compared to the control stored at 4 °C after 16 days. Chang et al. (2011) assessing the antioxidative properties of a chitosan–glucose Maillard reaction product and its effect on pork qualities

during refrigerated storage reported a reduction of 1.2 log CFU/g for mesophilic bacteria and 2.5 log CFU/g of *Pseudomonas* counts compared to the control sample. Kanatt et al. (2008) reported that the addition of a chitosan–glucose complex in lamb meat had the ability to reduce the number of spoilage organisms and thereby increase the shelf life. Thus, these results confirm that the modification of LMWC did not impact significantly on original antimicrobial activity since the microbiological quality of goat sausages were similar to that obtained with the non-modified LMWC, except for psychrophilic bacteria.

3.2. Physico-chemical analysis

3.2.1. Proximate composition and pH

Table 2 shows the effect of addition of LMWC and LMWC-Glc on the chemical composition and pH of fresh goat sausages. On day 0 the moisture, protein, fat and ash content were similar to those reported by Amaral et al. (2016) in fresh goat meat sausages manufactured with 12.5% of fat and added of 2% (w/w) de chitosan (LMWC), which found values of 65.52, 17.96, 13.93 and 2.07, respectively for moisture, protein, fat and ash. In our study, during storage at 4 °C the moisture content in fresh goat sausages decreased along the time ($p < 0.05$), probably due to the loss of water during storage since the casing used was water-permeable according the manufacturer. The loss in moisture content during cold storage of sausage could be referred to moisture vapor migration from the surfaces of sausage samples as a result of difference in water vapor pressure with the surrounding cold air (El-Nashi et al. 2015). Silva et al. (2014) to assess the shelf-life for cooked goat blood sausages also observed a decrease in moisture value. Regarding to chitosan and its derivative, the addition promoted a slight decrease in the moisture content when compared with the samples control

($p < 0.05$). This fact is due to the ability of chitosan to absorb water by hydrogen bonding through its hydroxyl and amine groups (Sormoli et al., 2012).

The results obtained for the protein, fat and ash content were more affected by storage time ($p < 0.05$) than the chitosans addition on formulation. These parameters during storage increased for all samples, which can be explained by the occurrence of concentration due to the reduction of moisture. Similar behavior was reported by El-Nashi et al. (2015) in beef sausage prepared with different concentrations of pomegranate peels powder during cold storage at 4 ± 2 °C for 12 days.

On the other hand, the pH values were affected ($p < 0.05$) by addition of chitosan and its derivative, as well as by the storage time. The chitosan and derivative chitosan increased the pH of the sausages when compared to the control sample (without LMWC), however the increase with LMWC-Glc lower, and at day 0 was not significant. This, can be attributed to the basic nature of chitosan (Georgantelis et al., 2007; Sayas-Barberá et al., 2011), promoted by the amino groups present. Amaral et al. (2016) in fresh goat sausage and Sayas-Barberá et al. (2011) in pork model burgers also reported that the addition of chitosan caused an increase in the pH. With respect to storage, the pH value reduced in all samples, although the reduction with LMWC-Glu was the least. Silva et al. (2014) in cooked goat blood sausage found similar behavior and reported that this indicates the possible development of lactic acid bacteria, which are microorganisms responsible for the decrease in pH, however not selective monitored in this study.

3.2.2. TBARS values

TBARS analysis had been most widely used for assessing the lipid oxidative status of meat (Qin et al. 2013; Falowo et al. 2014) and meat products. Meat becomes susceptible to

oxidative deterioration due to high concentrations of unsaturated lipids, heme pigments, metal catalysts and a range of oxidizing agents in the muscle tissue (Falowo et al. 2014). Oxidation of lipid and auto-oxidation are one of the major causes of quality deterioration and reduction of shelf life of meat products (El-Nashi et al., 2015). This reaction involves the loss of essential fatty acids and vitamins and the generation of toxic compounds as the malondialdehyde (MDA), as well as, affects sensory traits of product, causing flavor, color and texture deterioration (Estévez et al., 2005). With the aim of ensuring an extension of the shelf life of these kind of products, various synthetic additives are widely used in the food industry (Jayasena & Jo, 2013). However, their use has been restricted because of possible health risks and toxicity (Jayasena & Jo, 2013; Oliveira et al., 2012). In this respect, the chitosan due to their antioxidant activity has attracted especial interest from the meat industry as a potential natural food preservative (Shahidi et al., 1999). Similarly, studies have indicated that the chitosan–glucose complex showed superior antioxidant activity as compared to chitosan/glucose alone (Kanatt et al., 2008).

The results of TBARS analyses of fresh goat sausages during refrigerated storage are presented in Fig 1. Lipid oxidation increased proportionally with the storage period being always more intense in the control samples and derivative of chitosan (LMWC-Glc), both did not present statistically significant differences ($p > 0.05$), than in the samples added chitosan (LMWC). The sausages with non-modified chitosan reduced in mean the MDA values in 26.65% on day 7, 38.10% on day 14 and 43.63% after 21 days compared to control sample and derivative of chitosan, which has no potential antioxidant under the conditions of this study. Therefore, the results indicated that the chitosan may inhibit the development of lipid oxidation during storage at 4 °C. Shahidi et al. (1999) explained that chitosan would chelate the free irons, which are released from hemoproteins during heat processing or storage, and thus inhibit the lipid oxidation of products. Similar behavior was also observed

by Amaral et al. (2016) in fresh goat sausages prepared with different levels of fat (5, 12.5 and 20%), which reported that the addition of 2% (w/w) of chitosan can result in better quality and longer shelf life concerning lipid oxidation profile. The null effect of LMWC-Glc could be related to the fact that amino groups are compromised with glucose linkage not exerting the expected effect.

3.2.3. Color measurement

The L^* , a^* and b^* value of fresh goat sausages during storage is shown in figure 2. The initial L^* value on day 0 were slightly lower on sample treated than in control samples, but with statistical significance only in the sample added with derivative of chitosan (LMWC-Glc) (Fig 2a). The increase in L^* during the first day could be related to oxidation increasing metmyoglobin concentration (Sayas-Barberá et al., 2011). During storage, the L^* value reduced for all samples, remaining lower in samples with added LMWC and LMWC-Glc than the control sample ($p < 0.05$). This behavior could be due to the water binding ability of chitosan (Shahidi et al., 1999) and of derivative chitosan (Rui Matute et al., 2013), being in agreement with Fernandez-Lopez et al. (2000), which indicated that the lightness (L^*) reduces with increasing in water holding capacity (WHC).

The redness (a^*) values of fresh goat sausage decreased during refrigerated storage ($p < 0.05$) in all samples (Fig 2b). However, the decrease was more intense in the samples without LMWC and with LMWC-Glc. Amaral et al. (2016) reported a similar effect of LMWC on the a^* value of sausages, causing a more red surface in fresh goat sausage. Similar results were also obtained for pork meat products with chitosan (Darmadji & Izumimoto, 1994, Sayas-Barberá et al., 2011, and Amaral et al., 2015). This effect may be explained by ability

of chitosan in chelate iron ions of meat hemoproteins during heat processing or storage Georgantelis et al. (2007).

The b^* (yellowness) values also decreased with storage time (Fig 2c). Lekjin (2016) reported that the decrease in the parameters a^* and b^* could be associated with oxidative changes related to increases in TBARS causing pale color of the samples. In our study, the reduction of b^* was always lower in samples with LMWC, which can be explained by its antioxidant effect (Yen et al., 2008), and always higher in the control sample and added of LMWC-Glc, corroborating their higher values of TBARS discussed before. The natural color of the derivative of chitosan may also have been responsible for increasing the b^* values. Youn et al. (1999) reported increasing values of b^* and suggested that the natural color of chitosan affected on the surface color of sausage. Jo et al. (2001) reported that the addition of chitosan oligomer increased the color b^* value of pork sausages during storage. However, the impact on color is not evident on sensory evaluation section.

3.2.4. Fatty acid profile and cholesterol

Fatty acid profile of examined sausages is shown in Table 3. In general, the fatty acid content was not significantly affected ($p > 0.05$) by the addition of LMWC and LMWC-Glc. But, both sausages showed fatty acid content with slightly increase, while in control samples the sum of fatty acids slightly reduced during storage at 4 °C. The main fatty acids present in the fresh goat sausage were palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linolenic (C18:2) acid. A similar result was reported by Silva et al. (2013) in the smoked goat chorizo. They reported that these acids together represented approximately 93.20% of the total fatty acid composition. In our study, the highest percentage of monounsaturated fatty acids was oleic acid (~39%), which is distinguished for its high concentration in goat meat and pork

fat (Bragagnolo and Rodriguez-Amaya, 2002). Linoleic acid (C18:2) stood out among the polyunsaturated fatty acids at 12%. This fatty acid, is considered essential to the human body due to its specific physiological functions, having a role in the prevention of cardiovascular, autoimmune and inflammatory diseases (Thautwein, 2001).

After 21 days of storage, saturated fatty acid sum was significantly different ($p < 0.05$) for sausages treated with LMWC (44.09 mg/g) comparing to sausage with LMWC-Glc (31.86 mg/g) and control (25.92 mg/g). Monounsaturated and polyunsaturated fatty acid sums in the end of storage were higher ($p < 0.05$) in sausage with LMWC (41.48 mg/g and 12.95 mg/g, respectively) and LMWC-Glc (31.30 mg/g and 10.88 mg/g, respectively) compared to control sample (23.89 mg/g and 7.87 mg/g, respectively). The oleic acid (C18:1) content for control sausage, decreased during storage ($p < 0.05$), while for sausages with LMWC and LMWC-Glc the value did not change significantly during storage ($p > 0.05$). This decrease may be due to oxidative phenomena (Krkic et al., 2013). Thus, showed than LMWC and LMWC-Glc may have prevented the oxidation of oleic acid. Similar behavior was reported by Krkic et al., (2013) to assess the effect of chitosan coating on fatty acid profile of dry fermented sausage. These authors also found higher unsaturated and polyunsaturated fatty acid sums in the end of storage for the treated sample with chitosan than in control sausage.

The PUFA/SFA ratio is one of the main parameters used to assess the nutritional quality of the lipid fractions of foods (Leite et al. 2015; Silva et al. 2013). The ratio of PUFA:SFA recommended is between 0.4 and 0.5 by British Department of Health (1994). In our study, the fresh goat sausages showed mean ratios of 0.31. These results were similar to those reported by Leite et al. (2015) in goat meat sausages manufactured with different pork fat levels, which reported ratios between 0.13 and 0.33. The percentage of desirable fatty acids (DFA) was defined by Rhee (1992) as follows: PUFA+ MUFA+C18:0. The DFA values of

fresh goat sausages added with LMWC was slightly higher than control sausages and LMWC-Glc (data not shown). Therefore, confirming the antioxidant potential of chitosan, resulting in the preservation of the fatty acid profile.

The cholesterol content (Table 3) on day 0 was not affected ($p > 0.05$) by the addition of chitosan (60.88 mg/100g) and derivative chitosan (56.04 mg/100g), presenting results similar to control sample (60.04 mg/100g). During storage, the cholesterol content increased significantly for all samples ($p < 0.05$), being slightly higher ($p < 0.05$) in sausages with chitosan (87.63 mg/100g) and derivative chitosan (85.79 mg/100g) compared with the control samples (85.57 mg/100g) after 21 days of storage at 4 °C. This behavior, can be attributed the concentration of the fat content due to decrease of the moisture, which was significantly lower in treated samples than control sample, as described before.

3.2.4. Water retention capacity

One important attribute of sausage and other meat products is the ability to hold moisture and other juices in the product both before and after treatment (Yang et al. 2007). The water retention capacity (Table 2) of cooking goat sausages was affected by addition of chitosan and by the storage period ($p < 0.05$). The values for the treated samples with LMWC ($98.58 \pm 1.70\%$) and LMWC-Glc ($96.47 \pm 0.80\%$) ($p > 0.05$) was higher, at day 0, than the control samples ($95.50 \pm 1.90\%$), but with statistical significance only in the sample added of LMWC when compared with the control sample (without LMWC and LMWC-Glc). Similar behavior was reported by Amaral et al. (2016) in cooked goat sausage prepared with different amounts of fat (5, 12.5 and 20%) and 2% of chitosan, which found values of 98.00 ± 0.51 to 107.58 ± 0.22 and 87.49 ± 0.20 to 104.47 ± 0.50 for samples added with the chitosan and control sample, respectively. Jiang et al. (2012) reported that chitosan–glucose complex was

more effective to inhibit water loss in shiitake mushroom stored at 4 ± 1 °C for 16 days when compared with chitosan or glucose alone. This effect is due to water binding ability of chitosan (Sayas-Barberá et al., 2011). After 21 days of storage, water retention capacity increased for all samples, being always greater in the sausages with chitosan (LMWC and LMWC-Glc) than in control sample. Ayadi et al. (2007) reported similar behavior in turkey meat sausages with carrageenan and indicated that with the reduction in pH, the water holding capacity of proteins decreases which leads to an exudation of water outside the product during storage. Therefore, the chitosan and the chitosan derivative by having water retention capacity provide slightly higher yields of the samples.

3.3.5. Texture profile analysis

In a meat system, texture profile analysis is used to evaluate not only the consumer desirability of the products but also the structural integrity of the protein matrix (Souissi et al., 2016). The results obtained from cooked goat sausage texture analysis are shown in Fig. 3. Between the tested parameters, the hardness is one of more relevant markers and represents the maximum force required to compress the sample (Viana et al., 2003). The hardness of the different formulations increased slightly with the addition of LMWC and LMWC-Glc compared with the control sample (Fig. 3a). This increase in hardness can be explained by the fact that chitosan have ability to act as binder, thus favoring the formation of a stronger gel (Kachanechai et al., 2008) promoting a more stable structure. The increase in hardness by adding of chitosan has been reported in other studies with pork sausage (Amaral et al., 2015; García et al., 2010; Lin & Chao, 2001), as well as, in goat sausage (Amaral et al., 2016).

After 21 days of storage at 4 °C, the hardness of cooked goat sausage increased significantly ($p < 0.05$), with this increase being significantly higher in the sausage added of LMWC and LMWC-Glc than in the control sample. These effects can be explained by several factors, such as: loss of water during storage (Andrés, et al. 2006; Ganhão et al., 2010; Khalil, 2000), as described before, due to the stabilizations of chitosan linkages with matrix components at refrigerated temperature (Amaral, et al., 2016), as well as to protein-protein interaction (Andrés et al., 2006).

The addition of LMWC and LMWC-Glc in sausages also caused an increase in gumminess values (Fig. 3b) in relation to control samples. This parameter has direct dependence on result of hardness, thus justifying the similar behavior, increasing with the addition of chitosans and storage time. Gumminess is the force necessary to disintegrate a semi-solid state of the sample until swallowing (Viana et al., 2003). The other textural parameters in general were not affected by the addition of the chitosan and derivative chitosan ($p > 0.05$). García et al. (2010) in pork sausage and Amaral et al. (2016) in cooked goat sausage also reported that chitosan addition did not affect significantly the results of the texture profile analysis.

3.3. Sensory analysis

The results of sensory evaluation of cooked goat sausage are presented in Table 4. In general, all attributes showed a similar behavior, with slight decreasing acceptance ($p < 0.05$) throughout storage time. On the initial 0-day of storage, the appearance (visual homogeneity), odor (goat meat), flavour (rancid), texture (softness and juiciness) and overall acceptability scores did not differ significantly between the treatments ($p > 0.05$). After 14 day of storage at 4° C the odor of goat meat significantly reduced irrespective of treatment

($p > 0.05$). Similarly, the scores of appearance reduced over time, but was found lower visual homogeneity in the sample control ($p < 0.05$) than in samples treated with LMWC and LMWC-Glc. This effect can be justified by addition of chitosan, which may allow a sausage with a more stable structure (Amaral et al., 2015). Sayas-Barberá et al. (2011) and Soutos et al. (2008) reported that burgers and fresh pork sausage, respectively, containing chitosan presented the best visual appearance than the control sample.

The rancid flavor after 7 d of storage increased significantly ($p < 0.05$), as expected, being higher in control samples and containing LMWC-Glc than sausage added of LMWC, corroborating the result of TBARS analysis. This result can be explained by the antioxidant activity of chitosan. Similar behavior was reported by Soutos et al. (2008) in fresh pork sausage stored at 4 °C for 28 d containing 1% of chitosan. They indicated that the lower MDA values explain the reduction of rancid flavour, thus causing a more acceptable odour and taste in treated sample than the control samples.

Regarding the texture, LMWC and LMWC-Glc showed positive results, producing higher, numerically, softness and juiciness compared to the control sample, although no significant differences ($p > 0.05$) were observed between treatments after 7 days of storage. This behavior can be explained by water retention capacity displayed by the chitosan, as described before. Lin and Chao (2001) in reduced-fat Chinese-style sausage also reported scores slightly higher of juiciness for treatments containing chitosan and demonstrated that this addition resulted in no detrimental effect on textural properties.

Finally, the decrease in the overall acceptance after storage for 14 days, observed on all treatments ($p > 0.05$), was probably due to the reduction of goat meat odor and increase rancid flavor. Chang et al. (2011), also reported decrease in the overall acceptance and justify with the increased of off-odour on studies to assess the effect of chitosan–glucose Maillard

reaction product on pork qualities during refrigerated storage. Thus, confirming the results shown here.

4. Conclusions

The chitosan and chitosan–glucose maillard reaction product showed similar behavior, but the use of chitosan resulted in superior antioxidant activity than its derivative, considering the higher reduction of the values of TBARS and higher content of unsaturated and polyunsaturated fatty acids. However, the derivative of chitosan provided greater red color (a^*) and lower hardness than chitosan. In relation to sausages, the results of this study showed that derivative chitosan can also be used effectively to extend the shelf-life of fresh goat sausages stored under chill conditions as previously obtained for chitosan, because was observed reduction of microbial growth and lipid oxidation in treated samples, when compared with the control sample. Moreover, the fresh goat sausages added with derivative chitosan resulted in best technological quality as observed for chitosan, since it causes an increase in the stability, by ability to bind water and fat, a firmer texture, improvement in the red color, and preserved the fatty acid profile, without negative influences on the sensory qualities of the samples. Therefore, although it is necessary to conduct further studies, the use of 2% (w/w) chitosan or derivative chitosan, accomplish the requirements of the EFSA, can add value to goat meat allowing to obtain a low fat sausage with improved quality and potential health benefits.

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LEGENDS OF FIGURES

Figure 1. Evaluation of lipid oxidation in fresh goat sausages prepared with 2% (w/w) of chitosan (LMWC) and 2% (w/w) of chitosan-glucose derivative (LMWC-Glc) stored at 4 °C for 21 days.

Figure 2. Evaluation of **(a)** lightness (L^*), **(b)** redness/greenness (a^*) and **(c)** yellowness/blueness (b^*) in fresh goat sausages prepared with 2% (w/w) of chitosan (LMWC) and 2% (w/w) of chitosan-glucose derivative (LMWC-Glc) stored at 4 °C for 21 days.

Figure 3. Evaluation of **(a)** Hardness (N) and **(b)** Gumminess (N) in fresh goat sausages prepared with 2% (w/w) of chitosan (LMWC) and 2% (w/w) of chitosan-glucose derivative (LMWC-Glc) stored at 4 °C for 21 days.

FIGURES

Figure 1.

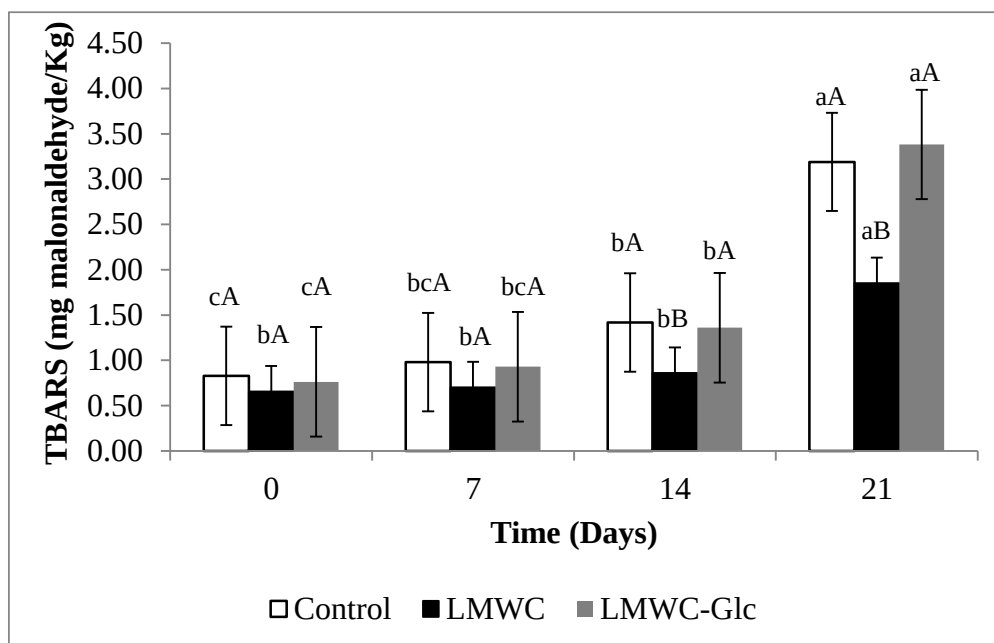


Figure 2.

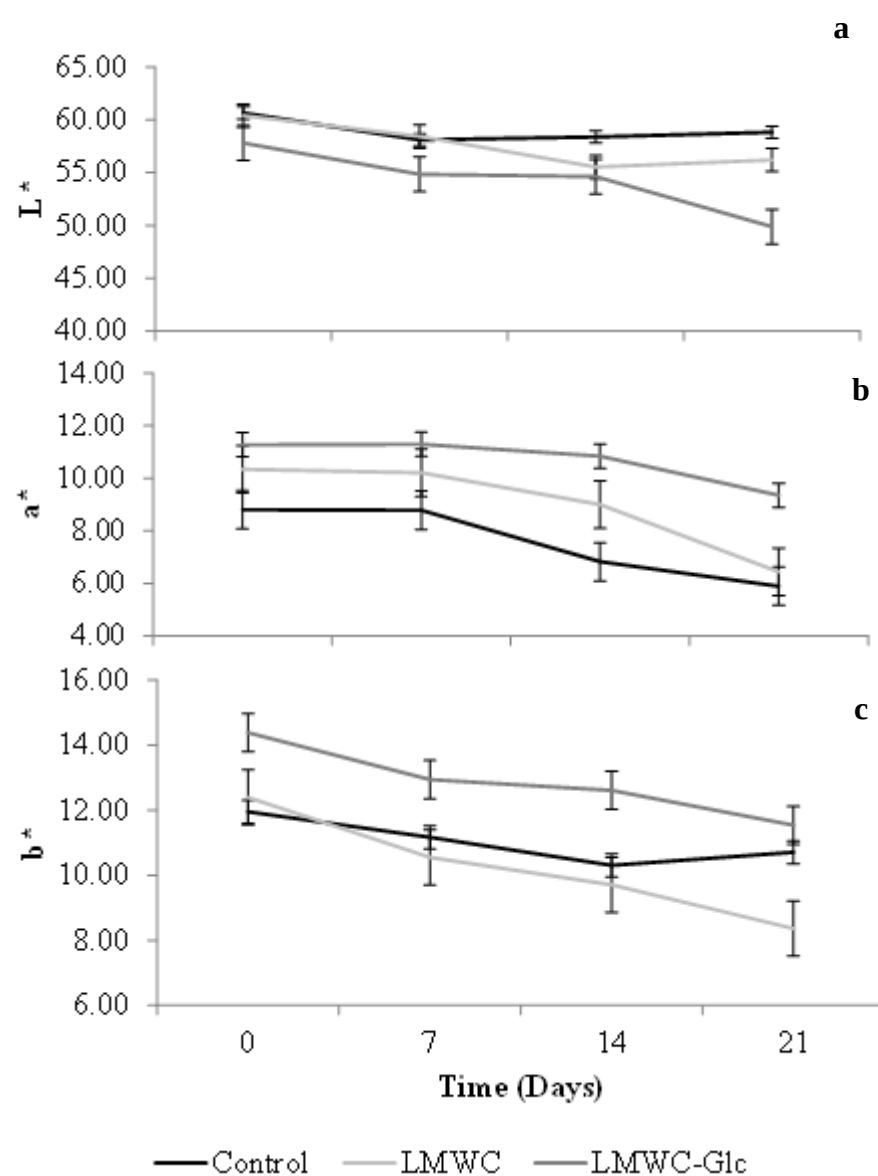
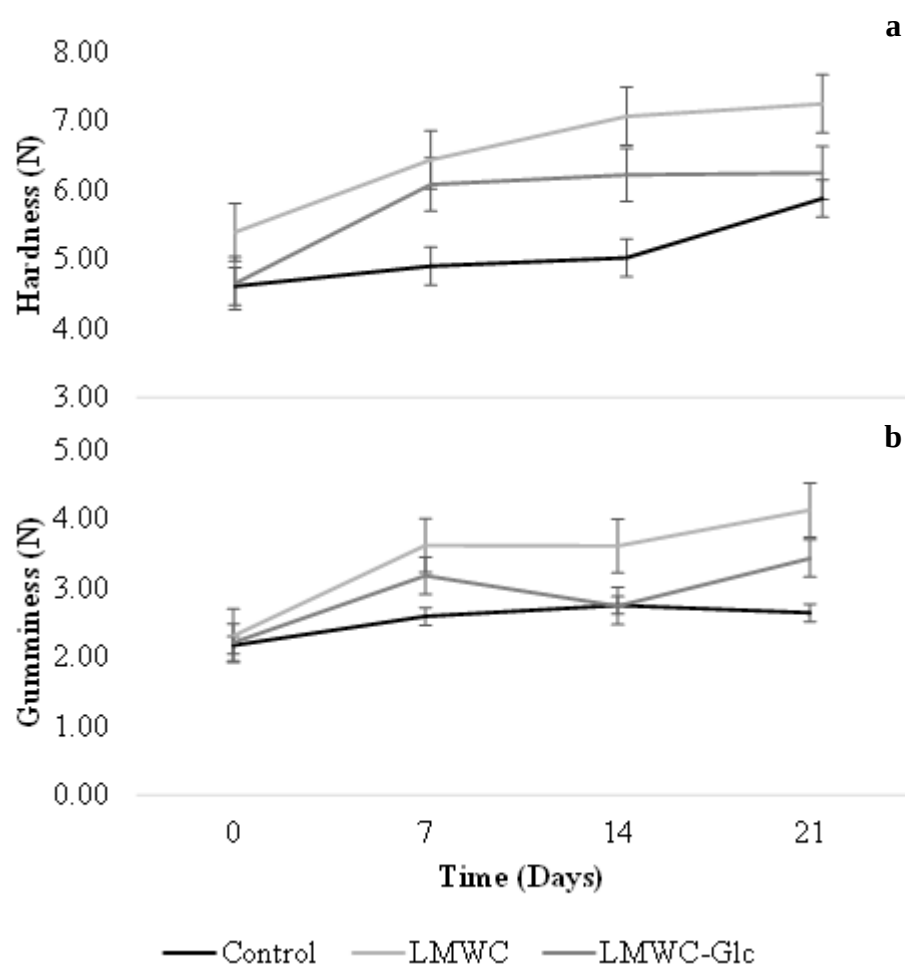


Figure 3.

TABLES

Table 1. Microbial counts (Log CFU/g) obtained for fresh pork sausages with 2% (w/w) of chitosan (LMWC) and 2% (w/w) of chitosan-glucose derivative (LMWC-Glc) stored at 4 °C for 21 days.

Variables	Treatments	Storage period (Days)*			
		0	7	14	21
Mesophilic (log10CFU/g)	Control	6.28±0.03 ^{dA}	7.52±0.02 ^{cA}	8.39±0.03 ^{bA}	9.21±0.08 ^{aA}
	LMWC	6.10±0.05 ^{dB}	7.03±0.03 ^{cC}	8.13±0.11 ^{bB}	8.46±0.04 ^{aB}
	LMWC-Glc	6.03±0.10 ^{dB}	7.22±0.06 ^{cB}	8.28±0.08 ^{bA}	8.41±0.05 ^{aB}
Psychrotrophic (log10CFU/g)	Control	5.15±0.08 ^{dB}	5.92±0.04 ^{cA}	7.21±0.15 ^{bA}	7.40±0.04 ^{aA}
	LMWC	4.87±0.10 ^{cC}	5.65±0.04 ^{bB}	6.75±0.02 ^{aB}	6.82±0.02 ^{aB}
	LMWC-Glc	5.36±0.05 ^{dA}	5.75±0.02 ^{cB}	6.72±0.02 ^{bB}	6.88±0.04 ^{aB}
Moulds and Yeasts (log10CFU/g)	Control	6.27±0.02 ^{cA}	7.63±0.03 ^{bA}	9.22±0.07 ^{aA}	9.13±0.03 ^{aA}
	LMWC	6.03±0.07 ^{dB}	7.30±0.03 ^{cC}	8.33±0.10 ^{bB}	8.79±0.07 ^{aB}
	LMWC-Glc	6.11±0.08 ^{dB}	7.42±0.02 ^{cB}	8.43±0.03 ^{bB}	8.55±0.07 ^{aC}
Enterobacteriaceae (log10CFU/g)	Control	5.77±0.02 ^{dA}	7.80±0.04 ^{cA}	8.65±0.11 ^{bA}	8.91±0.03 ^{aA}
	LMWC	5.54±0.03 ^{dB}	7.38±0.01 ^{cB}	8.17±0.03 ^{bB}	8.68±0.11 ^{aB}
	LMWC-Glc	5.61±0.04 ^{dB}	7.13±0.02 ^{cC}	8.13±0.03 ^{bB}	8.53±0.06 ^{aC}

*Different letters (a–b) in the same row differ significantly ($P < 0.05$) in time. Different letters (A–B) in the same column differ significantly ($P < 0.05$) in samples.

Table 2. Proximate composition obtained for fresh goat sausages and water retention capacity (WRC) calculated for cooked goat sausages prepared with 2% (w/w) of chitosan (LMWC) and 2% (w/w) of chitosan-glucose derivative (LMWC-Glc) stored at 4 °C for 21 days.

Variables	Treatments	Storage period (Days)*			
		0	7	14	21
Moisture (g/100g)	Control	69.63±0.45 ^{aA}	69.43±0.66 ^{aA}	67.55±0.41 ^{bA}	66.55±0.44 ^{cA}
	LMWC	66.47±0.73 ^{aC}	65.35±0.24 ^{bB}	64.97±0.54 ^{bB}	64.92±0.23 ^{bB}
	LMWC-Glc	67.51±0.53 ^{aB}	65.23±0.70 ^{bB}	64.29±0.30 ^{cC}	64.67±0.30 ^{bcB}
Protein (g/100g)	Control	18.09±0.66 ^{bB}	18.69±0.33 ^{bB}	21.03±0.45 ^{aA}	20.94±0.49 ^{aB}
	LMWC	19.92±0.53 ^{bA}	19.48±0.22 ^{bA}	21.52±0.32 ^{aA}	22.01±0.28 ^{aA}
	LMWC-Glc	18.21±0.30 ^{cB}	19.90±0.46 ^{bA}	21.37±0.40 ^{aA}	21.33±0.45 ^{aB}
Fat (g/100g)	Control	9.41±0.62 ^{cA}	10.67±0.33 ^{abB}	11.07±0.41 ^{aA}	9.99±0.40 ^{bcA}
	LMWC	9.45±0.51 ^{cA}	11.58±0.55 ^{aA}	11.40±0.51 ^{abA}	10.51±0.50 ^{bA}
	LMWC-Glc	9.30±0.34 ^{cA}	10.82±0.61 ^{bAB}	11.77±0.40 ^{aA}	10.54±0.67 ^{bA}
Ash (g/100g)	Control	2.21±0.05 ^{aA}	2.18±0.11 ^{aA}	2.23±0.14 ^{aA}	2.26±0.39 ^{aA}
	LMWC	2.19±0.05 ^{aA}	2.14±0.17 ^{aA}	2.26±0.05 ^{aA}	2.30±0.13 ^{aA}
	LMWC-Glc	2.10±0.29 ^{aA}	2.21±0.09 ^{aA}	2.23±0.11 ^{aA}	2.33±0.05 ^{aA}
pH	Control	6.75±0.03 ^{aB}	6.21±0.02 ^{bc}	6.16±0.01 ^{bB}	6.22±0.03 ^{bB}
	LMWC	6.91±0.14 ^{aA}	6.66±0.02 ^{bA}	6.48±0.04 ^{cA}	6.51±0.07 ^{cA}
	LMWC-Glc	6.76±0.17 ^{aB}	6.53±0.03 ^{bcB}	6.43±0.04 ^{cA}	6.54±0.02 ^{bA}
WRC (%) ^a	Control	95.50±1.90 ^{bB}	95.62±1.70 ^{bB}	97.80±1.47 ^{abB}	98.28±0.65 ^{aB}
	LMWC	98.58±1.70 ^{bA}	99.22±2.00 ^{abA}	101.00±2.27 ^{abA}	101.32±1.02 ^{aA}
	LMWC-Glc	96.47±0.80 ^{bAB}	98.53±2.90 ^{abA}	100.02±1.53 ^{aAB}	100.10±0.35 ^{aAB}

*Different letters (a–b) in the same row differ significantly ($P < 0.05$) in time. Different letters (A–B) in the same column differ significantly ($P < 0.05$) in samples.

Table 3. Fatty acid profile and cholesterol content in fresh goat sausages prepared with 2% (w/w) of chitosan (LMWC) and 2% (w/w) of chitosan-glucose derivative (LMWC-Glc) stored at 4 °C for 21 days.

Fatty acids (mg/100g)	Tratamento	Storage period (Days)*			
		0	7	14	21
C14:0	Control	1.67±0.07 ^{aA}	1.97±0.24 ^{aA}	2.09±0.15 ^{aA}	1.42±0.21 ^{aA}
	LMWC	1.68±0.09 ^{aA}	2.28±0.29 ^{aA}	2.98±1.47 ^{aA}	2.52±0.12 ^{aA}
	LMWC-Glc	1.60±0.15 ^{aA}	1.77±0.36 ^{aA}	2.18±0.20 ^{aA}	1.99±0.21 ^{aA}
C15:0	Control	0.32±0.01 ^{aA}	0.27±0.10 ^{aA}	0.29±0.01 ^{aB}	0.28±0.02 ^{aA}
	LMWC	0.28±0.03 ^{bA}	0.27±0.12 ^{bA}	0.73±0.18 ^{aA}	0.44±0.04 ^{bA}
	LMWC-Glc	0.28±0.03 ^{aA}	0.25±0.02 ^{aA}	0.35±0.04 ^{aB}	0.30±0.02 ^{aA}
C16:0	Control	17.89±0.53 ^{aA}	23.03±0.85 ^{aA}	25.15±1.73 ^{aA}	15.25±0.63 ^{aA}
	LMWC	18.78±0.22 ^{aA}	26.54±0.18 ^{aA}	30.01±0.74 ^{aA}	25.86±0.85 ^{aA}
	LMWC-Glc	17.35±1.66 ^{aA}	19.39±0.55 ^{aA}	21.88±2.27 ^{aA}	19.73±1.55 ^{aA}
C16:1	Control	1.87±0.04 ^{aA}	1.20±0.17 ^{abA}	0.43±0.03 ^{bB}	0.33±0.01 ^{bB}
	LMWC	0.34±0.01 ^{bB}	1.99±0.25 ^{aA}	2.39±1.06 ^{aA}	2.00±0.12 ^{aA}
	LMWC-Glc	0.33±0.02 ^{bB}	1.02±1.00 ^{abA}	1.76±0.22 ^{aA}	1.66±0.17 ^{aA}
C18:0	Control	9.57±0.22 ^{abA}	12.95±0.13 ^{abA}	14.14±0.93 ^{aA}	8.39±1.72 ^{bB}
	LMWC	10.35±0.29 ^{aA}	14.71±0.35 ^{aA}	13.25±0.14 ^{aA}	14.27±0.17 ^{aA}
	LMWC-Glc	9.85±0.11 ^{aA}	10.92±0.90 ^{aA}	12.09±1.18 ^{aA}	9.51±1.39 ^{aB}
C18:1n9c	Control	26.98±0.39 ^{abA}	34.66±0.07 ^{aA}	37.19±0.82 ^{aA}	22.27±0.64 ^{bB}
	LMWC	28.57±0.03 ^{aA}	39.05±2.07 ^{aA}	38.26±1.72 ^{aA}	37.35±0.53 ^{aA}
	LMWC-Glc	26.23±0.95 ^{aA}	28.93±2.58 ^{aA}	32.05±1.68 ^{aA}	28.01±0.09 ^{aAB}
C18:1c11	Control	1.51±0.03 ^{abA}	1.92±0.58 ^{abA}	2.10±0.26 ^{aA}	1.29±0.21 ^{bB}
	LMWC	1.57±0.03 ^{aA}	1.59±0.65 ^{aA}	1.67±0.17 ^{aA}	2.14±0.53 ^{aA}
	LMWC-Glc	1.43±1.31 ^{aA}	1.60±0.56 ^{aA}	1.79±0.21 ^{aA}	1.64±0.99 ^{aAB}
C18:2n6c	Control	8.62±0.27 ^{bcA}	10.80±0.03 ^{abAB}	11.76±0.39 ^{aA}	7.18±1.14 ^{cB}
	LMWC	8.81±0.00 ^{bA}	12.23±1.41 ^{aA}	10.03±0.04 ^{abA}	11.86±0.05 ^{aA}
	LMWC-Glc	7.97±0.13 ^{aA}	8.94±0.35 ^{aB}	10.16±1.32 ^{aA}	9.55±0.01 ^{aAB}
C20:0	Control	0.12±0.00 ^{aA}	0.15±0.63 ^{aA}	0.16±0.07 ^{aA}	0.10±0.02 ^{aA}
	LMWC	0.13±0.17 ^{aA}	0.16±0.01 ^{aA}	0.15±0.00 ^{aA}	0.16±0.49 ^{aA}
	LMWC-Glc	0.12±0.74 ^{aA}	0.13±1.97 ^{aA}	0.15±0.31 ^{aA}	0.10±0.05 ^{aA}
C18:3n3	Control	0.49±0.00 ^{aA}	0.59±0.00 ^{aA}	0.64±0.01 ^{aA}	0.44±0.03 ^{aA}
	LMWC	0.53±0.01 ^{aA}	0.65±0.10 ^{aA}	0.82±0.33 ^{aA}	0.67±0.00 ^{aA}
	LMWC-Glc	0.48±0.01 ^{aA}	0.50±0.02 ^{aA}	0.60±0.01 ^{aA}	0.55±0.00 ^{aA}
C20:2	Control	0.26±0.00 ^{aA}	0.33±0.013 ^{aA}	0.36±0.08 ^{aA}	0.22±0.00 ^{aA}
	LMWC	0.29±0.17 ^{aA}	0.37±0.03 ^{aA}	0.23±0.15 ^{aA}	0.38±0.04 ^{aA}
	LMWC-Glc	0.26±0.04 ^{aA}	0.28±0.09 ^{aA}	0.32±0.09 ^{aA}	0.28±0.06 ^{aA}
C20:4n6	Control	0.44±0.02 ^{abA}	0.67±0.01 ^{aA}	0.35±0.45 ^{abA}	0.02±0.03 ^{bA}
	LMWC	0.43±0.00 ^{abA}	0.65±0.11 ^{aA}	0.75±0.27 ^{aA}	0.04±0.01 ^{bA}
	LMWC-Glc	0.41±0.02 ^{aA}	0.48±0.05 ^{aA}	0.83±0.03 ^{aA}	0.50±0.03 ^{aA}

C23:0	Control	0.51±0.00 ^{aA}	0.11±0.01 ^{aA}	0.15±0.15 ^{aA}	0.49±0.04 ^{aAB}
	LMWC	0.52±0.08 ^{abA}	0.02±0.02 ^{bA}	0.04±0.02 ^{bA}	0.83±0.06 ^{aA}
	LMWC-Glc	0.56±0.04 ^{aA}	0.12±0.10 ^{aA}	0.43±0.54 ^{aA}	0.23±0.30 ^{aB}
Σ SFA	Control	30.08±0.61 ^{aA}	38.49±1.00 ^{aA}	41.97±3.73 ^{aA}	25.92±4.56 ^{aB}
	LMWC	31.75±0.13 ^{aA}	43.99±2.88 ^{aA}	47.16±2.19 ^{aA}	44.09±0.89 ^{aA}
	LMWC-Glc	29.76±2.84 ^{aA}	32.58±0.68 ^{aA}	37.07±4.25 ^{aA}	31.86±2.10 ^{aAB}
Σ MUFA	Control	30.36±0.39 ^{aA}	37.77±0.78 ^{aA}	39.72±3.06 ^{aA}	23.89±3.83 ^{aB}
	LMWC	30.48±0.03 ^{aA}	42.63±2.58 ^{aA}	42.32±4.61 ^{aA}	41.48±2.61 ^{aA}
	LMWC-Glc	27.98±0.47 ^{aA}	31.55±0.94 ^{aA}	35.60±4.13 ^{aA}	31.30±1.65 ^{aAB}
Σ PUFA	Control	9.82±0.30 ^{bcA}	12.39±0.67 ^{abAB}	13.10±0.00 ^{aA}	7.87±1.18 ^{cB}
	LMWC	10.05±0.18 ^{bA}	13.90±1.66 ^{aA}	11.84±0.50 ^{abA}	12.95±0.54 ^{abA}
	LMWC-Glc	9.12±0.82 ^{aA}	10.20±2.22 ^{aB}	11.91±1.12 ^{aA}	10.88±1.11 ^{aA}
MUFA/SFA	Control	1.01	0.98	0.95	0.92
	LMWC	0.96	0.97	0.90	0.94
	LMWC-Glc	0.94	0.97	0.96	0.98
PUFA/SFA	Control	0.33	0.32	0.31	0.30
	LMWC	0.32	0.32	0.25	0.29
	LMWC-Glc	0.31	0.31	0.32	0.34
Cholesterol	Control	60,04±0,88 ^{cA}	62,71±1,75 ^{cA}	70,99±1,86 ^{bA}	85,57±3,48 ^{aA}
	LMWC	60,88±0,88 ^{cA}	59,64±0,44 ^{cA}	70,06±1,75 ^{bA}	87,63±1,22 ^{aA}
	LMWC-Glc	56,04±0,44 ^{cA}	61,34±1,31 ^{bcA}	64,32±1,86 ^{bB}	85,79±1,82 ^{aA}

*Different letters (a–b) in the same row differ significantly ($P < 0.05$) in time. Different letters (A–B) in the same row differ significantly ($P < 0.05$) in samples.

ΣSFA: Sum of saturated fatty acids.

ΣMUFA: Sum of Monounsaturated Fatty Acids.

ΣPUFA: Sum of polyunsaturated fatty acids.

M/S: MUFA:SFA

P/S: PUFA:SFA

Table 4. Sensory parameters evaluated in cooked goat sausages prepared with 2% (w/w) of chitosan (LMWC) and 2% (w/w) of chitosan-glucose derivative (LMWC-Glc) stored at 4 °C for 21 days using a scale of 9 cm.

Variables	Treatments	Storage period (Days)*		
		0	7	14
Homogeneity	Control	6.57±0.84 ^{aA}	6.14±0.96 ^{aA}	5.02±0.97 ^{bB}
	LMWC	6.75±1.35 ^{aA}	6.76±0.98 ^{aA}	6.68±1.31 ^{aA}
	LMWC-Glc	7.14±1.09 ^{aA}	6.57±1.72 ^{aA}	6.48±1.98 ^{aA}
Goat meat flavour	Control	6.61±1.16 ^{aA}	2.57±1.00 ^{bC}	1.67±0.84 ^{bA}
	LMWC	6.81±1.11 ^{aA}	5.80±1.65 ^{aA}	1.88±1.35 ^{bA}
	LMWC-Glc	6.41±1.16 ^{aA}	4.39±1.23 ^{bB}	1.73±0.97 ^{cA}
Rancid flavor	Control	0.11±0.12 ^{bA}	0.71±0.77 ^{aAB}	ND
	LMWC	0.11±0.18 ^{bA}	0.33±0.54 ^{aB}	ND
	LMWC-Glc	0.11±0.16 ^{bA}	0.88±1.05 ^{aA}	ND
Juiciness	Control	5.53±1.74 ^{bA}	5.57±1.57 ^{aA}	ND
	LMWC	6.48±0.83 ^{aA}	6.57±0.64 ^{aA}	ND
	LMWC-Glc	6.49±0.59 ^{aA}	6.48±0.81 ^{aA}	ND
Succulence	Control	6.19±1.82 ^{aA}	5.90±2.37 ^{bA}	ND
	LMWC	6.97±2.27 ^{aA}	6.88±0.86 ^{aA}	ND
	LMWC-Glc	7.32±1.82 ^{aA}	6.64±1.18 ^{aA}	ND
Overall acceptance	Control	6.69±2.06 ^{aA}	5.06±2.32 ^{aA}	1.26±1.40 ^{bA}
	LMWC	7.02±2.64 ^{aA}	5.33±2.59 ^{aA}	1.61±1.25 ^{bA}
	LMWC-Glc	6.06±3.01 ^{aA}	5.59±2.06 ^{aA}	1.40±1.61 ^{bA}

*Different letters (a–b) in the same row differ significantly ($P < 0.05$) in time. Different letters (A–B) in the same column differ significantly ($P < 0.05$) in samples.

ND: not determined

6. CONCLUSÕES GERAIS

Os resultados obtidos indicaram que independente das salsichas serem elaboradas com carne suína ou caprina, a incorporação de 2% de quitosana é tecnologicamente viável, permitindo a elaboração de um produto com teor de gordura reduzido, além de atender as recomendações da EFSA (ingestão de 3g/dia de quitosana em 3 salsichas) quanto a redução do colesterol sérico.

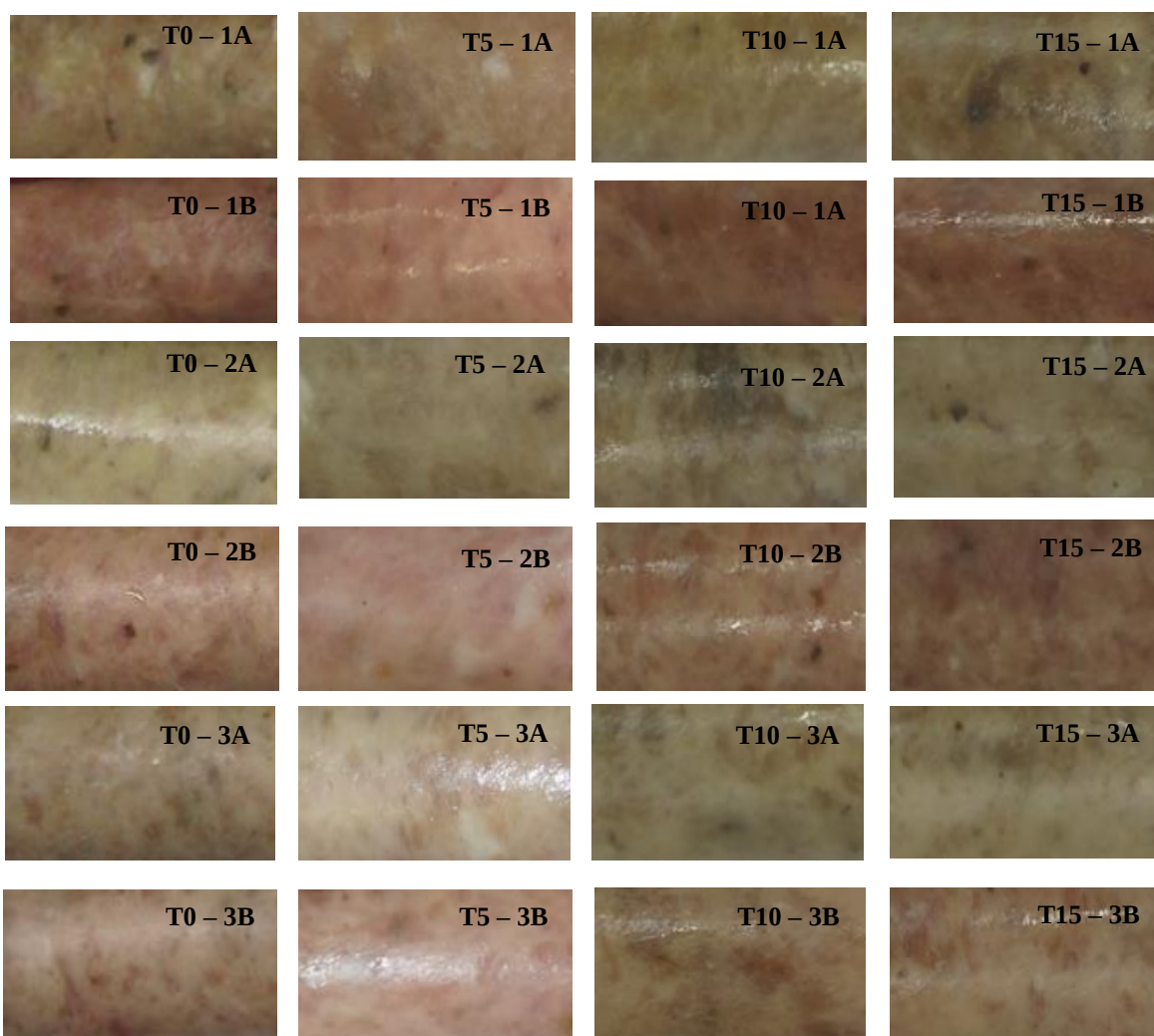
Além do potencial funcional, a quitosana e o derivado da quitosana proporcionaram aumento da estabilidade das salsichas tratadas, uma vez que provocaram a redução do crescimento microbiológico quando comparadas a amostra controle. Além disso, proporcionaram uma melhoria na cor vermelha, resultando em efeitos positivos na aparência, uma emulsão mais estável, pela maior capacidade de ligar água e gordura, e uma textura mais firme por aumentar as forças de compressão, sem afetar negativamente as propriedades sensoriais das salsichas.

A quitosana e o produto da reação de Maillard (quitosana-glicose) mostraram um comportamento semelhante, mas o uso da quitosana resultou em maior atividade antioxidante do que o seu derivado, considerando a maior redução dos valores de TBARS e maior teor de ácidos graxos insaturados e poli-insaturados. Entretanto, o derivado da quitosana promoveu maior cor vermelha (a^*) e menor dureza que a quitosana.

Por fim, a utilização da quitosana na fabricação de salsichas caprina foi estudada pela primeira vez e os resultados indicaram que a quitosana e o derivado da quitosana apresentaram potencial interessante para serem utilizados na elaboração de produtos cárneos caprinos, uma vez que podem proporcionar maior qualidade e vida de prateleira, agregar valor à matéria-prima de baixo valor comercial, além de permitir a obtenção de um produto funcional com alegação de saúde e redução de gordura.

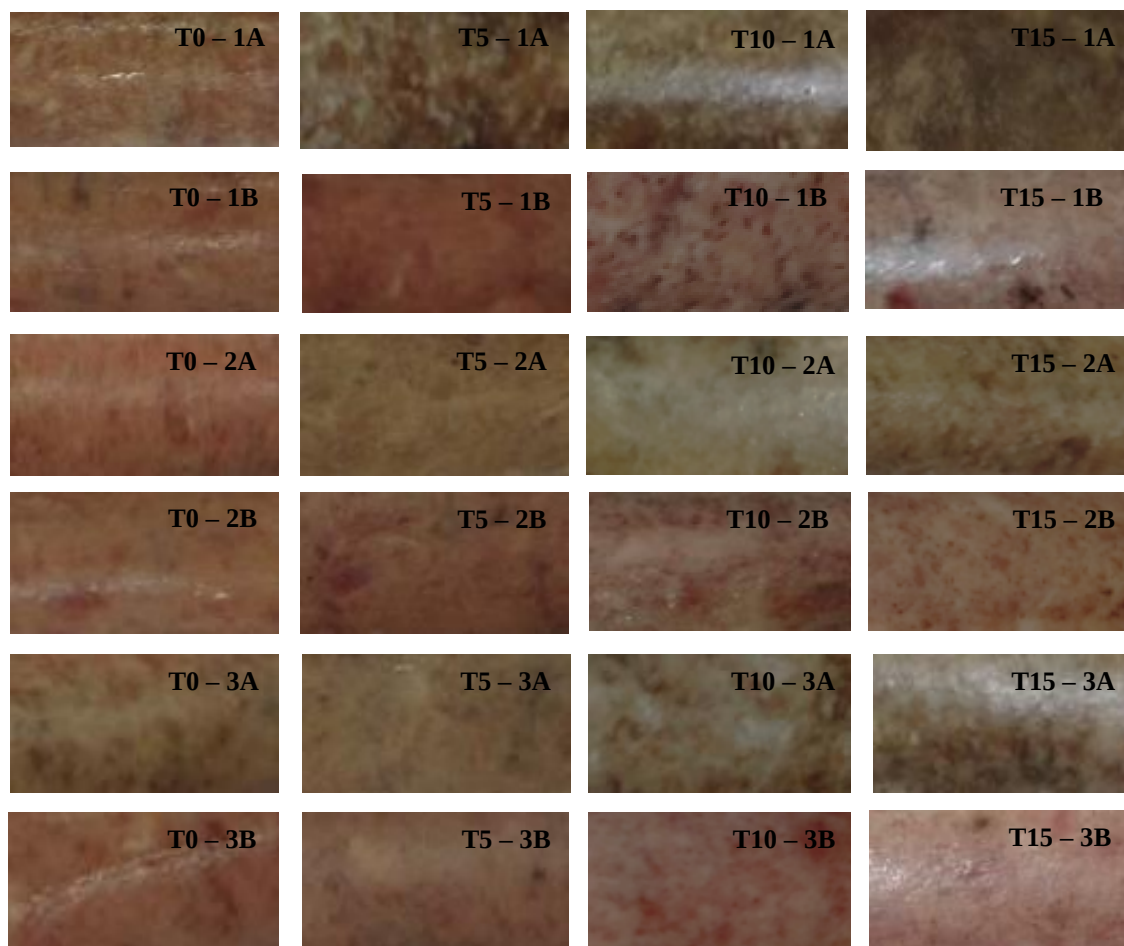
APÊNDICES

A - Mudanças da cor durante o armazenamento a 4 °C da salsicha fresca de porco - Experimento 1



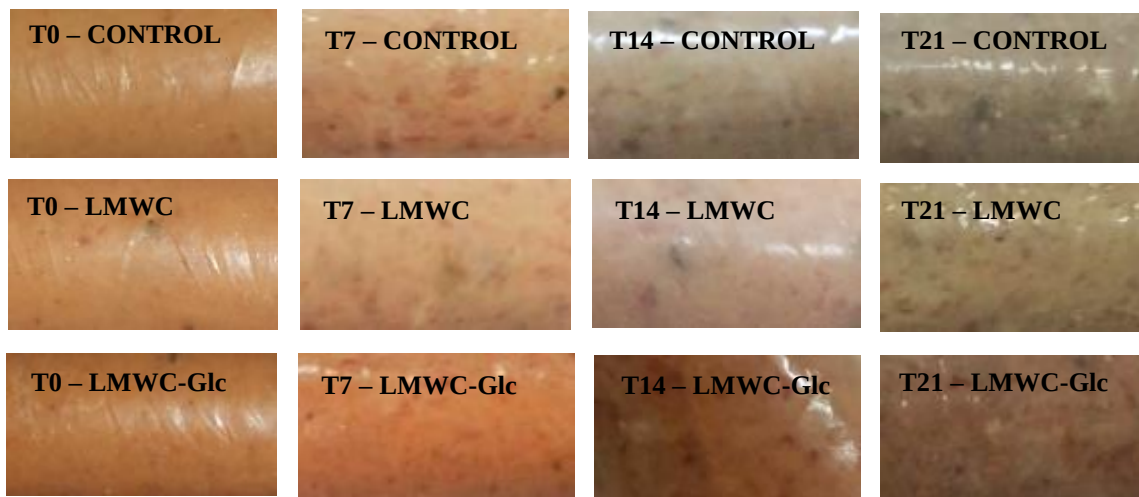
Mudança de cor das salsichas fresca de porco com 2% de quitosana (amostras A) e sem quitosana (amostras B), preparadas com diferentes quantidades de gordura: 5% (amostras 1), 12,5% (amostras 2) e 20% (amostras 3) e armazenada a 4 ° C durante 15 dias.

**B - Mudanças da cor durante o armazenamento a 4 °C da salsicha fresca de cabra-
Experimento 2**



Mudança de cor das salsichas fresca de cabra com 2% de quitosana (amostras A) e sem quitosana (amostras B), preparadas com diferentes quantidades de gordura: 5% (amostras 1), 12,5% (amostras 2) e 20% (amostras 3) e armazenada a 4 ° C durante 15 dias.

C - Mudanças da cor durante o armazenamento a 4 °C da salsicha caprina fresca - Experimento 3



Mudança de cor das salsichas fresca de cabra com 2% de LMWC ou LMWC-Glc e amostra controle sem quitosana e seu derivado armazenadas a 4 ° C durante 21 dias.

1 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Prezado (a) Senhor (a)

Esta pesquisa é sobre avaliação sensorial de salsicha elaborada com carne caprina e está sendo desenvolvida pela aluna de doutorado Deborah Silva do Amaral do Programa de Pós-Graduação em Ciências e Tecnologia de Alimentos / PPGCTA da Universidade Federal da Paraíba, sob a orientação da Prof^a Dr^a Marta Suely Madruga.

O objetivo deste estudo é avaliar o efeito da incorporação da quitosana e da quitosana modificada nos atributos microbiológicos, físico-químicos e sensoriais da salsicha elaborada com carne caprina. A finalidade deste trabalho é contribuir para avaliar o efeito da incorporação da quitosana e do derivado da quitosana nos atributos sensoriais da salsicha elaborada com carne caprina.

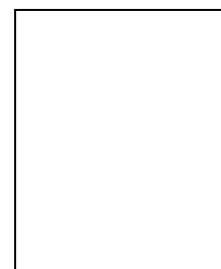
Para isto o participante será beneficiado com um brinde (não definido) no fim de cada seção, bem como será treinado na técnica sensorial Análise Descritiva Quantitativa.

Solicitamos a sua colaboração para participar do treinamento dos julgadores e da avaliação sensorial das amostras, como também solicitamos sua autorização para apresentar os resultados deste estudo em eventos da área de saúde e publicar em revista científica. Por ocasião da publicação dos resultados, seu nome será mantido em sigilo. Informamos que essa pesquisa não oferece riscos, previsíveis, para a sua saúde.

Esclarecemos que sua participação no estudo é voluntária e, portanto, o(a) senhor(a) não é obrigado(a) a fornecer as informações e/ou colaborar com as atividades solicitadas pelo Pesquisador(a). Caso decida não participar do estudo, ou resolver a qualquer momento desistir do mesmo, não sofrerá nenhum dano, nem haverá modificação na assistência que vem recebendo na Instituição. Os pesquisadores estarão a sua disposição para qualquer esclarecimento que considere necessário em qualquer etapa da pesquisa.

Diante do exposto, declaro que fui devidamente esclarecido(a) e dou o meu consentimento para participar da pesquisa e para publicação dos resultados. Estou ciente que receberei uma cópia desse documento.

Assinatura do Participante da Pesquisa
ou Responsável Legal



Espaço para impressão
dactiloscópica

Assinatura da Testemunha

Contato com o Pesquisador (a) Responsável:

Caso necessite de maiores informações sobre o presente estudo, favor ligar para o (a) pesquisador (a) Deborah Silva do Amaral.

Endereço (Setor de Trabalho): LAQA – Laboratório de Análises Físico-Química.

Telefone: (88) 96314612

Ou

Comitê de Ética em Pesquisa do CCS/UFPB – Cidade Universitária / Campus I

Bloco Arnaldo Tavares, sala 812 – Fone: (83) 3216-7791

Atenciosamente,

Assinatura do Pesquisador Responsável

Assinatura do Pesquisador Participante

2- QUESTIONÁRIO DE RECRUTAMENTO PARA ANÁLISE SENSORIAL

Nome: _____ Telefone: _____
E-mail: _____

Faixa etária: () Menos de 21 () 21-30 () 31-40 () 41-50 () 51-60 () acima de 61

Gênero: () Feminino () Masculino

Grau de escolaridade:

() Ensino Médio () Ensino Superior () Pós-Graduação

Com que frequência você consome carne caprina ou derivados?

() Todos os dias () 2 a 3 vezes por semana () 1 vez por semana () 1 a 2 vezes por mês

Consome salsicha: () Sim () Não

Fumante: () Sim () Não

Com que frequência você consome salsicha?

() Todos os dias () 2 a 3 vezes por semana () 1 vez por semana () 1 a 2 vezes por mês

Como consome salsicha?

Pão () Cuscuz () Inhame/macaxeira/batata doce () Outros ()

Quais: _____

Indique o quanto você aprecia cada um destes produtos

Produto	Gosto	Indiferente	Não Gosto
Salsicha	_____	_____	_____
Carne caprina	_____	_____	_____
Alimentos Funcionais	_____	_____	_____

Qual destas frases mais combina com seu perfil de consumo de gordura?

A redução da gordura no alimento não altera muito o sabor e a textura ()

Prefiro alimentos com baixo teor de gordura ()

Gosto de alimentos com alto teor de gordura ()

Existe algum alimento, condimento ou ingrediente pelo qual você apresenta intolerância, aversão ou alergia? Explique o motivo.

Você toma alguma medicação que afete seus sentidos, especialmente o paladar e o olfato? Se positivo, qual? _____

É hipertenso? () Sim () Não

Disponibilidade de horário para participar das seções de ADQ (Tem preferência por algum dia ou horário para as sessões?)
: _____

Existe algum dia ou horário no qual não possa participar das sessões de ADQ?

Você comeria produto cárneo enriquecido com fibra?

() Sim Qual motivo? _____

() Não

Obrigada!

3- TESTE TRIANGULAR

Nome: _____

Data: ____/____/____

Você está recebendo três amostras codificadas, sendo duas iguais e uma diferente.

Identifique com um círculo a amostra diferente.

Você considerou a amostra diferente por causa:

() da aparência () do sabor () do aroma () da textura

Comentários:

4 - TESTE DE RECONHECIMENTO DE GOSTOS

Nome: _____

Data: ____/____/____

Você está recebendo uma série de amostras apresentando os sabores básicos: **doce, salgado, amargo, ácido, umami**. Prove cada solução na ordem em que estão apresentadas. Descreva o gosto percebido, registrando sua percepção no quadro abaixo. Enxágue a boca após cada avaliação e aguarde alguns segundos para provar a próxima solução.

Nº da Amostra	Descrição do Gosto

Nº da Amostra	Descrição do Gosto

5 – HABILIDADES COM ESCALA

Nome: _____

Data: ____ / ____ / ____

Por favor, marque na linha à direita de cada figura, um trecho que indique a proporção da figura que foi coberta de preto. Não use régua, use apenas sua capacidade visual para avaliar.

a)



b)



c)



Sua vez:

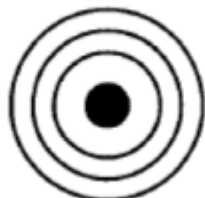
d)



e)



f)



g)



h)



6- TESTE DE TERMINOLOGIA DESCRITIVA

Nome: _____ Data: _____
____/____/____

Amostras: _____

SIMILARIDADES

DIFERENÇAS

Amostras: _____

SIMILARIDADES

DIFERENÇAS

Amostras: _____

SIMILARIDADES

DIFERENÇAS

7 – REFERÊNCIAS

	ATRIBUTO	DEFINIÇÃO	REFERÊNCIAS
APARÊNCIA	Homogeneidade/ presença de olhaduras	Quantidade de olhaduras (orifícios) de tamanho e forma irregulares, distribuídas nas salsichas.	POUCO: Queijo processado marca polenguinho tradicional MUITO: Queijo coalho artesanal
	Cor marron	Intensidade característica de carne caprina cozida, com variação de marrom claro a marrom escuro	FRACO: carne caprina cozida a 75°C FORTE: carne caprina grelhada a 75°C
	Suculência visual	Liberação de líquido que varia desde aparência seca a quantidade visível de líquido separado da salsicha	POUCO: torrada MUITO: queijo ricota
AROMA	De condimento	Aroma de condimento empregado tradicionalmente em salsicha tipo <i>hot-dog</i>	FRACO: nenhum FORTE: combinação de alho, cebola, pimenta preta e orégano
	De carne caprina	Aroma característico de carne caprina	FRACO: carne caprina cozida a 75°C FORTE: carne caprina grelhada a 75°C
	De ranço	Aroma de gordura oxidada	FRACO: Nenhum FORTE: Gordura oxidada
SABOR	Carne caprina	Sabor de carne caprina cozida	FRACO: carne caprina cozida a 75°C FORTE: carne caprina grelhada a 75°C
	Picante	Sensação de ardência (sabor apimentado) percebida com a amostra na boca.	POUCO: 0,1g de pimenta diluída em 100ml de água MUITO: 0,8g de pimenta diluída em 100ml de água
	Adstringente/ amargo	Sensação tátil de constrição das mucosas da boca pelo excesso de tanino (levemente amargo)	FRACO: Nenhum FORTE: solução de cafeína

	De ranço	Sabor de gordura oxidada	POUCO: queijo minas MUITO: gordura oxidada
	De condimento	Sabor de condimento empregado tradicionalmente em salsicha tipo <i>hot-dog</i>	FRACO: nenhum FORTE: combinação de alho, cebola, pimenta preta e orégano
	Salgado	Sensação associado a presença de sal	POUCO: solução com 0,2% de NaCl MUITO: solução com 0,7% de NaCl
TEXTURA	Maciez	Propriedade de textura que oferece pouca resistência à mastigação, variando de duro até macio	POUCO (DURA): Salsicha grelhada MUITO (MACIA): Kitut
	Suculência	Umidade liberada durante a mastigação.	POUCA: contra-filé bovino bem passado, a 80°C; MUITA: filé mignon mal passado a 65°C.
	Elasticidade	Após a compressão parcial da amostra com os dentes incisivos ou molares, sem rompê-la, observou-se o grau com que a amostra retornava a sua forma original.	POUCO: requeijão cremoso MUITO: <i>mashmallow</i>
	Mastigabilidade	Número de mastigações necessárias antes da deglutição	POUCO: pão bisnaguinha MUITA: bala de iogurte
AVALIAÇÃO GLOBAL		Reflete a total coerência em relação à avaliação feita pelos julgadores para cada um dos atributos	NENHUMA: total incoerência com os atributos avaliados; MUITA: total coerência com os atributos avaliados.

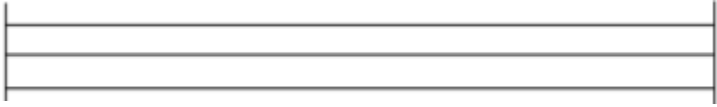
8- FICHA DE AVALIAÇÃO SENSORIAL DE SALSICHA CAPRINA

Nome: _____ Data ____/____/____

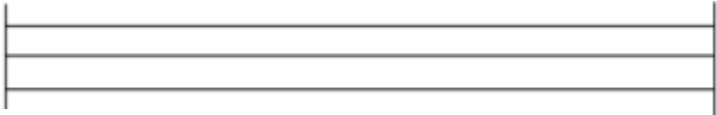
Por favor, serão avaliadas amostras de salsicha caprina de forma monádica, indique com um traço vertical o ponto que melhor reflete a sua resposta em relação aos atributos. Entre uma amostra e outra coma o biscoito e lave a boca com água.

APARÊNCIA

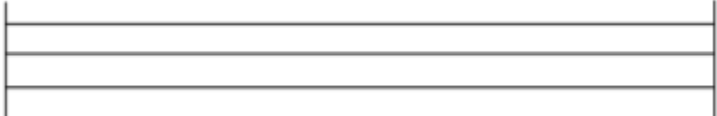
• Homogeneidade/ presença de olhaduras

 POUCO MUITA	AMOSTRAS
---	--

• Cor marron

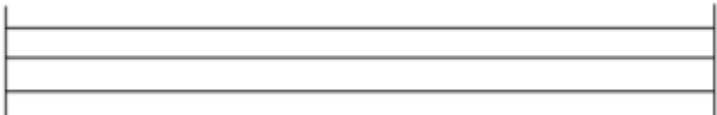
 FRACO FORTE	AMOSTRAS
---	--

• Suculência visual

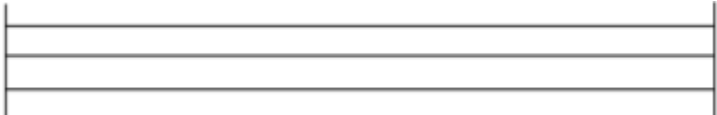
 POUCO MUITO	AMOSTRAS
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AROMA

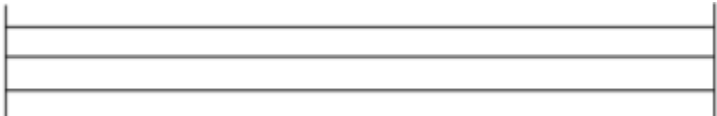
• De condimento

 NENHUM FORTE	AMOSTRAS
--	--

• De carne caprina

 FRACO FORTE	AMOSTRAS
---	--

• De ranço

 NENHUM FORTE	AMOSTRAS
--	--

SABOR

- Sabor característico de carne caprina

FRACO FORTE

AMOSTRAS

AMOSTRAS

- Picante

POUCO MUITO

- Adstringente/ amargo

FRACO FORTE

AMOSTRAS

- De ranço

POUCO MUITO

AMOSTRAS

- De condimento

FRACO FORTE

AMOSTRAS

- Salgado

POUCO MUITO

AMOSTRAS

TEXTURA

- Maciez

Pouco(DURA) Muito(MACIA)

AMOSTRAS

- Suculência

POUCA MUITA

AMOSTRAS

- **Elasticidade**

	POUCA	MUITA	AMOSTRAS
			
			
			

- **Mastigabilidade**

	POUCA	MUITA	AMOSTRAS
			
			
			

AVALIAÇÃO GLOBAL

	DESGOSTO EXTREMAMENTE	GOSTO EXTREMAMENTE	AMOSTRAS
			
			
			

ANEXO A: Comprovante de submissão do artigo 1

**USE OF CHITOSAN TO IMPROVE QUALITY AND
FUNCTIONALITY OF MEAT PRODUCTS: A REVIEW**

Journal:	<i>Critical Reviews in Food Science and Nutrition</i>
Manuscript ID	BFSN-2016-2243
Manuscript Type:	Review
Date Submitted by the Author:	10-Aug-2016
Complete List of Authors:	Amaral, Deborah; Federal University of Paraíba Cardelle Cobas, Alejandra; Universidade Catolica Portuguesa; Universidade Católica Portuguesa/Porto, Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia Madruga, Marta; Federal University of Paraíba Pintado, Manuela; Universidade Católica Portuguesa, CBQF/Escola Superior de Biotecnologia
Keywords:	meat, chitosan, food ingredient, functional foods, preservation

ANEXO B: Comprovante de submissão do artigo 4

Elsevier Editorial System(tm) for Journal of
Functional Foods
Manuscript Draft

Manuscript Number:

Title: LOW FAT GOAT MEAT SAUSAGE WITH CHITOSAN-GLUCOSE MAILLARD REACTION
PRODUCT: IMPACT ON QUALITY AND SHELF LIFE

Article Type: Full Length Article

Keywords: chitosan; chitosan-glucose; functional meat product; fat
reduction; Maillard reaction; cholesterol reduction

Corresponding Author: Dr. Manuela Pintado, Ph.D

Corresponding Author's Institution: CBQF/Escola Superior de
Biotecnologia, Universidade Católica Portuguesa

First Author: Deborah Amaral

Order of Authors: Deborah Amaral; Alejandra Cardelle-Cobas; Samara
Silva; Celina Dias; Naciza Arcanjo; Paulo Dalmás; Marta Madruga;
Manuela Pintado, Ph.D

Abstract: Low fat fresh goat sausages based on chitosan (LMWC) and derivative chitosan (LMWC-Glc) were developed aiming to produce a functional food product evaluating the effect of inclusion of these compounds on quality, stability and shelf life. Goat sausages (10% pork fat) were formulated with 2% (w/w) of LMWC or LMWC-Glc and stored at 4 °C during 21 days. Results indicated that incorporation of LMWC-Glc and LMWC is technologically feasible, originating a potential functional product to claim reduction of cholesterol, besides the positive impact on microbial safety and lipid oxidation. The modified LMWC (towards better solubility and lower adstringency) incorporated at 2% as functional ingredient showed lower antioxidant activity than the native chitosan, but maintained positive effect on antimicrobial, nutritional, textural and sensory performance. The results also proved that derivative chitosan may also be a promising strategy for improving the quality and extending the shelf life of low fat goat sausage.