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Camila Vespúcio Bis Souza

Inovação em embutidos cárneos fermentados:
Incorporação de micro-organismos probióticos e fibra prebiótica em formulações com teor reduzido de gordura.

São José do Rio Preto
2020

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Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Engenharia e Ciência de Alimentos, junto ao Programa de Pós-Graduação em Engenharia e Ciência de Alimentos, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de São José do Rio Preto.

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RESUMO

A reformulação de produtos cárneos fermentados tem se mostrado uma tendência para o segmento de alimentos mais saudáveis e é considerada um desafio para pesquisadores do mundo todo. A substituição parcial da gordura suína por fibras alimentares em embutidos cárneos fermentados tem minimizado os efeitos indesejáveis nas características tecnológicas e sensoriais causados pela redução de gordura, tais como aumento da dureza, maior perda de peso e menor aceitação pelos consumidores. Outra estratégia que tem se destacado é a incorporação de micro-organismos probióticos ou potencialmente probióticos (tais como, *L. rhmanosus* GG® e *L. casei*) no processamento de embutidos cárneos fermentados. A adição destas cepas de micro-organismos com propriedades funcionais pode auxiliar na inibição do crescimento da microbiota indesejada, reduzir a oxidação lipídica e acelerar as reações de proteólise, além de acarretar benefícios comprovados à saúde do consumidor, os quais envolvem a redução no colesterol, auxílio no funcionamento do sistema gastrointestinal e do sistema imunológico. Assim, o objetivo deste estudo foi reformular embutidos cárneos fermentados (salame tipo italiano e *Salchichón* espanhol) através da substituição parcial da gordura suínas por fibras prebióticas (frutooligossacarideos, inulina e alfa ciclodextrina) e incorporação de micro-organismos probióticos e avaliar as propriedades físico-químicas e sensoriais. Dentre as avaliações tecnológicas, pode-se destacar o perfil de aminoácidos livres, o comportamento do perfil de compostos voláteis, oxidação lipídica, perfil de textura e cor instrumental, além da aceitação sensorial de salame tipo italiano com teor reduzido de gordura e Salchichón espanhol. A adição de frutooligossacarídeos ou inulina até 2% resultou em uma melhora na aceitação sensorial de salame tipo italiano com teor reduzido de gordura suína. Outro resultado obtido foi a comprovação de que cepas potencialmente probióticas de *L. casei* podem ser utilizadas no processamento de salame tipo italiano com teor reduzido de gordura mantendo alta contagem de células viáveis (8 log UFC/g). A substituição parcial de gordura suína por frutooligossacarídeos e a adição de *Lactobacillus rhamanosus* GG resultou em uma redução de 29% no teor de gordura em um *Salchichón* Espanhol além de alterar o conteúdo total de compostos voláteis no produto final. Sendo assim, foi possível inovar na produção de embutidos cárneos fermentados com apelo mais saudável, mantendo atrativos sensoriais e tecnológicos utilizando estratégias promissoras.

Palavras–chave: Bactérias ácido láticas, Fibras prebióticas, Frutooligossacarídeos, Propriedades funcionais, Produtos cárneos fermentados.

ABSTRACT

The reformulation of fermented meat products has proved to be a trend for the healthier food segment and is considered a challenge for researchers around the world. The partial replacement of pork back fat with dietary fiber in fermented meat sausages has minimized the undesirable effects on technological and sensory characteristics caused by this reduction, such as increased hardness and weight loss, and decreased consumer acceptance. Another strategy that has received attention is the incorporation of probiotics or potentially probiotic microorganisms (such as *L. rhmanosus* GG® and *L. casei)* in the processing of fermented sausage. The addition of these microorganisms' strains with functional properties can assist in inhibiting the growth of unwanted microbiota, reduce lipid oxidation and accelerate proteolysis reactions, in addition to providing proven benefits to consumer health, which involve lowering cholesterol and helping gastrointestinal and immune systems functions. Thus, the the aim of this study was to reformulate fermented meat sausages (Italian type salami and Spanish Salchichón) by partially replacing swine fat with prebiotic fibers (fructooligosaccharides, inulin and alpha cyclodextrin) and incorporating probiotic microorganisms and evaluating the physicochemical properties and sensory. Among the evaluated technological characteristics, it is noteworthy the free amino acid profile, the behavior of the volatile compound profile, lipid oxidation, texture profile, and instrumental color, in addition to the sensory acceptance of low-fat Italian type salami and Spanish Salchichón. The addition of fructooligosaccharides or inulin up to 2% resulted in an improvement in the sensory acceptance of Italian type salami with reduced content of pork back fat. Another result obtained was the proof that potentially probiotic *L. casei* strains can be used in the processing of low-fat Italian type salami maintaining a high viable cell count (8 log CFU/g) in the final product. The partial replacement of pork back fat by fructooligosaccharides and the addition of *Lactobacillus rhamanosus* GG resulted in a 29% reduction in the fat content in a Spanish *Salchichón* and changed the total content of volatile organic compounds. Thus, it was possible to innovate in the production of healthier fermented meat sausages with an appeal to the consumer due to their sensory, functional, and technological properties, using promising strategies.

Keywords: Lactic acid bacteria, Prebiotic fibers, Fructooligosaccharides, Functional properties, Fermented meat products.

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1. INTRODUÇÃO GERAL

A demanda por produtos alimentícios com características mais saudáveis e que mantenham o atrativo sensorial vem crescendo a cada dia. E esta tendência mundial na alimentação vem modificando a relação entre os consumidores e os produtos cárneos tradicionais. As estratégias mais utilizadas para produção de produtos cárneos mais saudáveis incluem: redução do nível de gordura, utilização de fibras alimentares como substituto de gordura, incorporação de cepas probióticas em embutidos fermentados, além da redução de aditivos na formulação (MENDOZA et al., 2001; BARRETTO et al., 2015; HAM et al., 2016; SELANI et al., 2016; TABANELLI et al., 2016; AFSHARI et al., 2017; PAVLI et al., 2020).

A redução no conteúdo de gordura de produto cárneo pode modificar propriedades importantes como aumentar a dureza, diminuir a intensidade da cor vermelha, diminuir o rendimento e, provavelmente, diminuir sua aceitação pelos consumidores. Assim, a indústria tem utilizado ingredientes que possam auxiliar na redução do conteúdo de gordura dos produtos cárneos tradicionais e que também agreguem benefícios nutricionais aos produtos cárneos, como o uso de fibras alimentares, fibras prebióticas e minerais, ou pela presença de compostos bioativos.

De acordo com (GIBSON et al., 2017), a fibra prebiótica é definida como um substrato que é utilizado seletivamente por micro-organismos hospedeiros que conferem um benefício a saúde do consumidor. Dentre os prebióticos mais estudados em produtos alimentícios, como substitutos de gordura, destacam-se inulina, frutooligossacarídeos e alfa-ciclodextrina (CÁRCERES et al., 2004; HUANG et al., 2011; SANTOS et al., 2012; CAVA et al., 2012; ÁLVAREZ & BARBUT, 2013; KEENAN et al., 2014; BARRETTO et al., 2015; AFSHARI et al., 2017; HENCK et al., 2019) os quais não são digeridos pelo trato gasto intestinal humano e são metabolizados por micro-organismos da flora intestinal (*Bifidobacterium* spp. and *Lactobacillus* spp.*)* resultando em ácidos graxos de cadeia curta (LANGA et al., 2019). Mendoza et al. (2001) afirmaram que a inulina pode ser considerada um excelente substituto de gordura em salames com teor de gordura reduzido, em função da melhora na textura final do produto quando comparado com o tratamento com redução de gordura sem utilização de substitutos de gordura, além da melhora no parâmetro sensorial de textura.

De acordo com Rouhi et al. (2013), a incorporação de fibras prebióticas em produtos cárneos fermentados, além de substituir a gordura, pode estimular o desenvolvimento dos micro-organismos probióticos e inibir o crescimento de patógenos em produtos cárneos, representando uma alternativa inovadora que pode ser usada pelas indústrias de processamento de carnes.

O mercado global de probióticos vem crescendo nos últimos anos em função do uso de cepas probióticas em diferentes tipos de produtos alimentícios (DE VUYST et al., 2008). Estima-se que os alimentos adicionados com probióticos representam 70% do mercado total de alimentos funcionais (TRIPATHI & GIRI, 2014) e os embutidos cárneos fermentados estão cada vez mais fazendo parte desta tendência (DECKER & PARK, 2010; WÓJCIAK et al., 2012; CAVALHEIRO et al., 2015; BLAIOTTA et al., 2018; NEFFE-SKOCINSKA et al., 2017).

Os probióticos são definidos como micro-organismos vivos que, quando administrados em quantidade adequada, conferem efeitos específicos e benéficos à saúde do consumidor (FAO/WHO, 2002) e estão incluídos entre os alimentos e/ou ingredientes alimentícios que podem apresentar alegação de propriedades funcionais ou de saúde (BRASIL, 2016). Para garantir os efeitos funcionais à saúde do hospedeiro, o micro-organismo probiótico deve ser capaz de crescer no produto, e sobreviver mantendo uma contagem de células viáveis suficiente até atingir o intestino. Um dos critérios mais importantes para a seleção das bactérias probióticas é a adesão à mucosa intestinal e às células epiteliais; essa adesão evita que as células benéficas sejam levadas pela motilidade do trato gastrointestinal e, assim, permite colonização temporária, modulação imunológica (GUIMARÃES et al., 2020).

Os probióticos podem fornecer uma série de efeitos benéficos para a saúde como proteção contra patógenos gastrointestinais, aumento do sistema imunológico, resistência à colonização, controle de doenças de vasos inflamatórios, equilíbrio da composição de microbiota intestinal, redução do nível de colesterol sérico e pressão sanguínea (TRIPATHI & GIRI, 2014). Estes benefícios são dependentes da cepa probiótica utilizada no processo de fermentação (REIS et al., 2011).

Os produtos cárneos fermentados apresentam condições favoráveis para veicular os micro-organismos probióticos em função da proteção das células bacterianas exercida pelos lipídeos, durante a passagem pelo trato gastrointestinal (ROUHI et al., 2013). Além disso, os embutidos fermentados são prontos para o consumo, atendendo também a demanda por praticidade e conveniência. Sendo assim, a combinação do uso de probióticos e a adição de fibras para a reformulação de embutido cárneo fermentado, com teor reduzido de gordura, pode trazer benefícios ao consumidor final, além de prover conhecimento que auxiliará no avanço científico da área de produtos cárneos.

2. OBJETIVOS

2.1. Objetivo geral

Avaliar o efeito da adição de fibras prebióticas e incorporação de cepas probióticas em produtos cárneos fermentados com redução parcial de gordura sobre as propriedades físico-químicas e sensoriais.

2.2. Objetivos específicos

Avaliar o efeito da substituição parcial de gordura suína por fibras alimentares prebióticas (inulina, frutooligossacarídeos (FOS) e alfa-ciclodextrina) em salame tipo italiano sobre as propriedades tecnológicas e sensoriais, utilizando delineamento de misturas.

Selecionar, a partir de testes *in vitro*, cepas probióticas de bactérias ácido láticas (BAL) (*Lactobacillus casei* SJRP) quanto à resistência nas condições do processamento de embutido cárneo fermentado.

Avaliar o efeito de cepas probióticas pré-selecionadas sobre o processamento de salame tipo italiano com teor reduzido de gordura adicionado de frutooligossacarídeos (FOS).

Avaliar o efeito sobre as características tecnológicas e sensoriais da adição de cepas probióticas comerciais em embutido fermentado espanhol “Salchichón” adicionado de frutooligossacarídeos (FOS) como substituto de gordura sobre formação de amino ácidos livres, parâmetros de textura, oxidação lipídica, cor instrumental e segurança microbiológica.

Avaliar o perfil dos compostos voláteis de um embutido fermentado espanhol “Salchichón” adicionado de frutooligossacarídeos (FOS) como substituto de gordura e cepas probióticas comerciais.

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4. ORGANIZAÇÃO DOS CAPÍTULOS

Este trabalho foi subdividido em cinco capítulos para um melhor entendimento dos assuntos abordados.

O capítulo 1 consiste em uma revisão bibliográfica intitulada “New Strategies for the development of innovative fermented meat products: a review of the incorporation of probiotics and dietary fibers” publicada no periódico *Food Reviews International*, volume 35, páginas 467-484, 2019. Neste capítulo, os autores relatam sobre as estratégias inovadoras adotadas pelos pesquisadores na área de produtos cárneos a fim de torna-lo mais saudáveis e atrativos aos consumidores atuais. Dentre as estratégias abordadas nesta revisão, destacam-se o uso de fibras alimentares como substitutos parciais de gordura animal e a utilização de micro-organismos probióticos como coadjuvantes na tecnologia produtiva de embutidos cárneos fermentados.

O capítulo 2 consiste em um artigo científico intitulado “Can dietary fiber improve the technological characteristics and sensory acceptance of low-fat Italian type salami? publicado no periódico *Journal of Food Science and Technology*. Este capítulo apresenta a substituição parcial de gordura suína em salame tipo italiano utilizando um design de mistura com três fibras alimentares diferentes. O objetivo desta etapa foi escolher uma formulação ótima em relação aos parâmetros tecnológicos e sensoriais. E a partir desta formulação realizar os trabalhos descritos nos capítulos 3, 4 e 5.

O capítulo 3 consiste em um artigo científico intitulado “Applicability of potentially probiotic *Lactobacillus casei* in low-fat Italian type salami: screening *in vitro* and technological evaluation” submetido no periódico *Meat Science*. Neste capítulo será apresentado um estudo de viabilidade de cepas potencialmente probióticas e a aplicação em um sistema modelo de salame tipo italiano.

O capítulo 4 consiste em um artigo científico intitulado “Impact of fructooligosaccharides and probiotic strains on the quality parameters of low-fat Spanish Salchichón” publicado no periódico *Meat Science,* v.159, 2020. Neste capítulo os autores avaliam as características tecnológicas e sensoriais de um embutido cárneo fermentado típico espanhol *Salchichón* reformulado comparado com uma formulação controle. Nesta etapa, a gordura suína foi parcialmente substituída por FOS e duas cepas comerciais de micro-organismos probióticos também foram adicionadas nas formulações (*Lactobacillus paracasei* e *Lactobacillus rhamanosus*).

O capítulo 5 consiste em um artigo científico publicado intitulado “Volatile profile of fermented sausages with commercial probiotic strains and fructooligosaccharides” publicado no periódico *Journal of Food Science and Technology*, 2019. Este capítulo apresenta-se uma continuação do capítulo 4 com ênfase no estudo do comportamento dos compostos voláteis do *Salchichón* quando submetido à reformulação.

Por fim, será apresentada a conclusão geral do trabalho realizado.

CAPÍTULO 1

Artigo Publicado no periódico *Food Reviews International*.

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New strategies for the development of innovative fermented meat products: a review of the incorporation of probiotics and dietary fibers.

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ABSTRACT- Over the last decades, consumer´s preference for and their attention to food products presented as healthy and with favorable nutritional information has increased significantly. In this line, both the addition of dietary fiber and the incorporation of probiotic strains in the elaboration of fermented meat products has been established as a useful tool for the development of healthy products. Thus, the aim of this review is to present an overview of the studies involving fermented meat products with added dietary fiber and probiotic microorganisms and, also, to discuss some of the challenges regarding the reformulation of this innovative product category.

Keywords: Fermented meat products; Prebiotic fibers, functional meat products; new food products; healthy.

1. INTRODUCTION

Meat is a highly nutritious food, rich in protein, essentials amino acids, vitamins (especially the B group vitamins) and minerals such as zinc and iron, which has formed part of the human diet from ancient times [1].

Over recent decades, traditional foods have been targeted for innovation. One area the food industry has shown an increased interest in is the development of healthier meat products. The strategies of innovative production can improve some high-value products, giving them an even higher value. The manufacturing of typical products has always been related to their technological evaluation [2]. This trend can be justified by the recent concerns about the regular consumption of meat due to its high content in saturated fats, which is associated with a higher risk of diseases such as obesity, heart disease and cancers [1, 3, 4].

The increased intention of consumers to buy healthier meat products with favorable nutritional information has led to the reformulation of some traditional meat products. From the consumer point of view, innovation should not lead to the loss of sensory characteristics of the traditional product [5]. Therefore, the testing of new strategies is essential to ensure a healthier meat product with good acceptability. The current growth of fermented meat products among meat products should be noted [6-10].

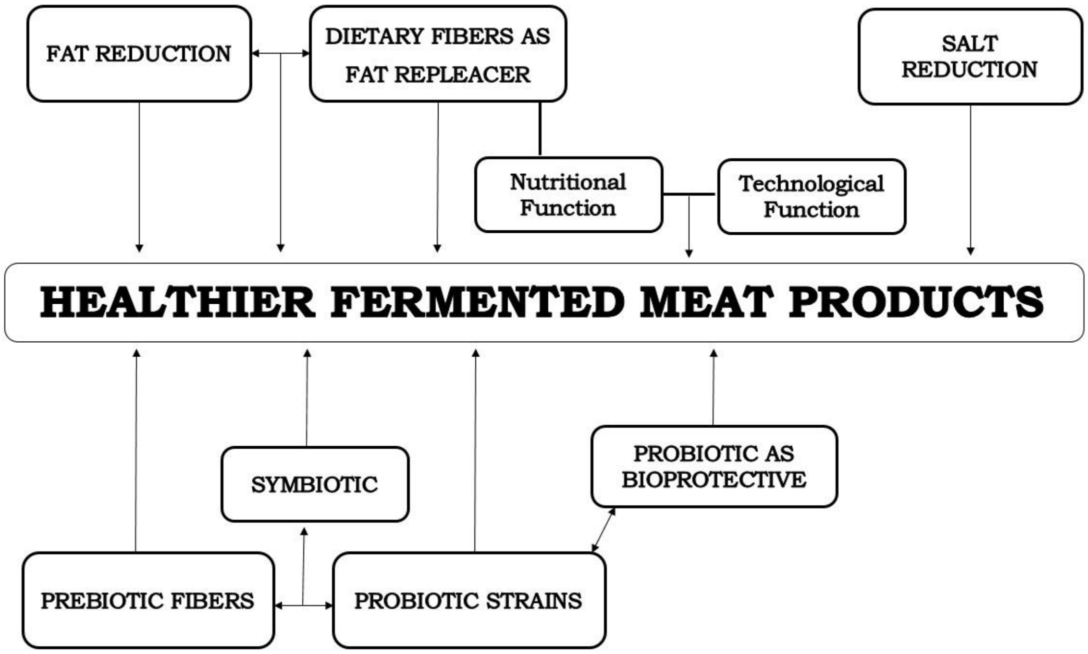
Although the technology of fermented meat production is old, it has gone through several important innovations throughout history [11]. The production of fermented meat products is a dynamic and complex process. Fermented meat products are found in different parts of the world and, in Europe, there is greater diversity and greater consumption of this type of meat product. The fermentation of the meat raw material is mainly carried out by lactic acid bacteria (LAB) and the acidic pH provides innumerable benefits for its conservation [12].

Among the most commonly used strategies to produce healthier meat products are: reduction of fat level, reduction of sodium level, use of dietary fiber as a fat substitute, incorporation of probiotic strains in fermented products and the reduction of additives in the formulation. Additionally, the use of novel non-thermal and thermal technologies in the manufacture of fermented meat products can improve the process efficiency and the safety and quality of products [13-20] (**Fig.1**). The incorporation of dietary fiber in meat products has been suggested for the reduction of the saturated fat level and also to increase the fiber content (nutritional function) and to increase the water holding capacity and the yield (technological function) [21].

For instance, the global market for probiotics has been growing in recent years due to their frequent use in the development of different types of food products [22, 23]. Food products with added probiotics account for approximately 70% of the total functional food market [24].

Probiotics are defined as “live strains of selected microorganisms which, when administered in an adequate amount, confer specific and beneficial effects on the health of the consumer” [25]. Probiotics have been associated with a beneficial effect on gastrointestinal, allergic and obesity diseases, insulin resistance syndrome and non-alcoholic fatty liver disease [26]. Moreover, they can provide several beneficial effects on health such as protection against gastrointestinal pathogens, enhancement of the immune system, control of inflammatory diseases, intestinal balance of microbiota composition, reduction of both serum cholesterol level and blood pressure [24]. However, these benefits will depend on the strain used in the fermentation process [27].

Figure 1. Commonly used strategies to produce healthier meat products



Fonte: Figura elaborada pelo autor.

Fermented meat products present favorable conditions for improved survival rates of probiotic microorganisms, due to the protective effect of lipids on bacterial cells passing through the gastrointestinal tract [28]. In addition, fermented sausages are products that are ready for consumption, this being a useful strategy to satisfy the growing demand for practicality and convenience [1].

On the other hand, another useful strategy to develop healthier meat products consists of the addition of dietary fiber. In this line, several authors have shown positive and promising results after incorporating dietary fiber in meat products in order to improve the meat functional properties as substitutes of animal fat. The small particles of dietary fiber have the ability to form gels [29], to increase yield [30] and to simulate the fat properties regarding texture parameters. In this context, short chain fibers such as inulin and fructooligosaccharides have been widely used as fat replacers in different meat products [31, 32].

The combination of probiotics and the addition of dietary fibers in order to develop new fermented meat products with reduced fat content can bring several benefits to the final consumer from a health point of view. This review aims to present different strategies which can be adopted by both food researchers and the food industry to develop innovative functional meat products, which have an improved nutritional profile (eg. saturated fat reduction).

2. HOW TO REFORMULATE MEAT PRODUCTS?

The fermentation of meat raw material provides numerous benefits for its preservation, the use of fermenting bacteria being necessary at this stage of processing. LAB are the most commonly used due to their acidification ability, which results in a lower pH in the system and also prevents the development of pathogenic microorganisms [1, 33]. In addition, the acidic pH of fermented meat improves the sensory parameters of the final product, such as texture, color and aromatic compounds, which directly enhance the sensorial acceptance [34].

The fermented sausages are characterized by their pungent and strong flavor. They are meat products made from pork, beef or a mixture of both. The characteristic flavor appears as a consequence of LAB fermentation, which promote the development of organic acids, such as lactic acid. The fermented sausages pH can vary from 4.6 to 5.2, depending on the type of sausage produced. Microbiological safety and sensorial quality of the final product are guaranteed by temperature control during fermentation, low pH, high sodium chloride concentration and the presence of sodium nitrite as a preservative [35].

The high fat content of fermented sausages contributes to their characteristic sensory characteristics, such as taste, texture, juiciness and appearance. Therefore, a significant reduction of fat content directly affects their acceptability [36]. The reduction in the saturated fat content of a meat product is related to a public health problem, since there is an association between the excessive consumption of saturated fat and an increase in the risk of heart disease and cancer. In this context, the addition of probiotic microorganisms and the reduction of fat content of meat products have been encouraged. Since meat and meat products are important sources of essential nutrients for the diet of the world population, the reformulation of these types of product has become of greater importance and an ongoing challenge [18].

Therefore, an increased interest has been shown in the consumption of healthier food products, which have reduced caloric value, fat content, sodium and sugar content. The reformulation strategies include the reduction in the amounts of these primary ingredients from the food matrix and the incorporation of beneficial compounds into traditional formulations, which can offer additional functional properties or health advantages to the products [12].

Moreover, with this strategy it is possible to address both the reduction of fat and the addition of probiotic microorganisms with their related beneficial effects on health.

2.1. Fat reduction and/or replacement

Due to the concern of consumers regarding the use of traditional meat products in their diet, researchers and food manufacturers have explored the use of new ingredients to meet such demand [14, 16]. Consumer preference for healthy and nutritious products has guided scientists and the food industry in developing new products [37-39].

Thus, in order to attend this demand for healthier products, several studies have been conducted to test new ingredients, new technologies and enrich traditional formulations with functional ingredients (mainly probiotic cultures and fibers) or reducing other components (nitrite, fat, sodium and sugar) [28, 40-42].

This reformulation is a huge challenge for the food industry, due to the need to maintain the sensorial and microbiological stability of the product [43, 44]. Fat contributes positively to the acceptance of meat [43, 45-47]. However, in order to reduce the harmful effects on health of high fat in meat products, it is necessary to incorporate non-meat ingredients, which perform technological and sensory functions similar to animal fat. Thus, the industry aims to use ingredients that can help reduce the fat content of traditional meat products and add nutritional benefits to meat products, such as the use of dietary fiber, prebiotic fibers, minerals or the presence of bioactive compounds [1, 48].

For instance, several authors reported positive results regarding the inclusion of dietary fibers, such as animal fat substitutes [14, 49-53], the use of probiotic microorganisms for the fermentation process [10, 28, 39, 40, 54-61] and enrichment of the traditional formulations with prebiotic fibers [62-64] to improve functional properties and make meat products healthier.

2.1.1. Prebiotic fibers as a fat substitute

Prebiotics are defined as non-digestible food ingredients with benefits in the host, due to their ability to stimulate the growth or selective activity of a group or a bacterial species resident in the colon [65]. Inulin, oligofructose, lactulose, galactooligosaccharides (GOS) and synthetic fructooligosaccharides (FOS) are examples of prebiotics.

These prebiotics have several common uses in food products, including fat replacement and as texturizing agents, among others [66]. Besides that, prebiotics fibers can also be used for their physiological features as a soluble dietary fiber.

Dietary fibers play an essential role in different physiological processes, such as disease prevention, maintenance of blood glucose levels and body weight as well as proper functioning of the intestine [16, 32, 67]. Additionally, dietary fiber fermentation by the gut microbiota results in the production of short chain fatty acids (SCFA), mainly acetate, propionate, and butyrate, which shift the host microbiota towards a more beneficial composition [68].

The incorporation of fibers into meat products can encourage healthier consumption habits, helping to reduce the incidence of diseases related to public health problems such as childhood obesity. The increase in dietary fiber intake has boosted the development of technological procedures for obtaining fiber concentrates from a range of raw materials, including industrial by-products [69].

The amount recommended by the U.S. Food and Drug Administration (FDA) for dietary fiber is 20 to 35 g per day, of which 75% should be of insoluble fibers and 25% of soluble fiber [70]. However, in industrialized countries, the consumption of dietary fiber is less than 25 g/person/day. One way to increase the amount of dietary fiber ingested in the diet without radically changing the consumption habit is to consume fiber-enriched foods [71]. A food product label can show the health claim as "high fiber content" if it contains at least 5 g of dietary fiber per portion of the food, while the product with a "fiber source" claim must contain at least 2.5 g of dietary fiber per serving of food [72].

The dietary fibers are considered fat substitutes with various applications in the meat products industry, because of their recognized physiological and technological properties [6, 73- 75]. The use of dietary fiber in meat products could constitute a useful alternative to fat replacers because it has several technological properties; it can improve texture, increase the ability to bind water, maintain a good yield and reduce the cost of new meat formulations [30, 44, 64, 76-78]. Some dietary fibers have a strong hygroscopic capacity, thus favoring increased water holding capacity and facilitating the absorption of fat, being able to absorb up to 20 times its weight in water [21].

Among the dietary fibers used as ingredients in meat products, the most notable are inulin, fructooligosaccharides, wheat fiber, rice bran, oat soluble fiber (β-glucan), insoluble oats, oat bran, soybean fiber, pea fiber, coconut fiber and fiber obtained from food industrial byproducts [14, 16, 52, 79, 80].

Wastes and/or by-products from the food industry are also being incorporated into meat products to minimize the effects of reducing the fat content of the formulation [81]. For instance, by-products (peel and pulp) from the industrialization of pineapple (1.5%) were added to beef burger obtaining a 10% reduction in fat content and effectively improving beef burger cooking characteristics such as yield and appearance. Moreover, the authors did not observe significant changes in the sensory acceptance of the final products compared to the control samples [16]. Moreover, dietary fiber, such as wheat fiber and oat fiber, can improve the stability of emulsions, facilitating the entrapment of fat along with the protein matrix. In this respect, in a study conducted by Barretto et al. [12], the authors observed good sensory acceptance of the product when dietary fiber was used as a substitute for 50% fat in the development of emulsified meat product.

Another study incorporated 1.5 and 3% of orange, beet, wheat, oat and pea fibers in salami as fat replacers. Samples with 1.5% of fruit and cereal fibers presented satisfactory sensory results, while samples with 3% had an increase in hardness and a significant reduction in sensorial properties. Additionally, the orange fiber product had similar sensory properties to the commercial product [82].

In another study, Campagnol et al. [83] evaluated the partial replacement of fat (40%) in fermented sausage by using 1 and 2% of soybean fiber. The technological characteristics and microbiological safety remained unaffected by the addition of soybean fiber. However, when 2% of soybean fiber was added, changes in the product texture were observed. Furthermore, there was a significant reduction in the volatile compounds related to lipid oxidation, as well as an increase in the release of volatile compounds related to amino acids.

Campagnol et al. [84] replaced the pork fat content of fermented sausage (75% and 100%) by a food fiber (amorphous cellulose fiber - Ztrim®). They found a reduction in the yield and lipid oxidation as well as a reduction in the sensorial acceptance of the final product. However, with a reduction of only 25 or 50%, no changes were observed in relation to the control samples. Moreover, the microbiological safety of the final product was maintained, independent of the modifications.

In a study carried out on beef burger with 10% fat reduction and addition of 13.45% soluble oat fiber [30], there was a reduction ($p \leq 0.05$) in water loss during cooking and an increase in water and fat retention, when compared to the control sample (20% fat). However, the addition of dietary fiber negatively affected the sensory acceptance of the sample.

Among prebiotic fibers, inulin and FOS are widely used due to their composition and functional characteristics. Inulin is the natural source of oligosaccharides from different plants such as chicory and shows important gelling properties [64]. Inulin can modify low-fat meat product texture, thus providing similar texture parameters to high fat meat products [13]. These unique properties presented by inulin justify its extensive use as a substitute for fat in different meat products with reduced fat content [85].

In relation to its functional characteristics, inulin is not absorbed by the small intestine but is fermented by the microorganisms present in the colon, which makes it a prebiotic dietary fiber. Thus, a fermented meat product with added inulin can be considered a functional food product [64, 66, 86]. Keenan et al. [85] observed a beneficial effect of inulin (0%, 3.12%, 6.23%, 7.79%, 9.35%, 12.47%, 18.70%) in preparing sausage, as it assisted in reducing water loss during cooking and increasing emulsion stability. However, it increased the hardness of the final products and negatively modified sensory quality.

The use of inulin, wheat fiber and oat fiber (3.5% and 7%) as fat substitutes in sausage was evaluated by Salazar, García and Selgas [87]. There was an increase in the hardness of the samples in relation to the controls. However, the addition of inulin in both proportions did not affect the texture parameters, observing increased influence on texture when the amount of fiber added to the food was increased.

Besides inulin, fructooligosaccharides (FOS) are also oligosaccharides, which are called unconventional sugars - components of plant origin and their biosynthesis occurs widely in nature. These compounds are present as energy reserves in innumerable plant species, many of which are used in foods, such as asparagus, beet, garlic, chicory, onion, wheat, barley, and rye [62].

In a study carried out on fermented sausage, the addition of up to 12% FOS as a fat substitute presented satisfactory results [75]. No change in the pH of the final product were observed after the addition of fat replacer. However, there was an increased yield compared to the control. In addition, FOS reduced approximately 40% fat content and 35% caloric value of the product and resulted in good sensorial acceptance at up to 12% substitution levels [88].

The addition of FOS as a fat replacer in baked fermented sausage (pepperoni) proved to be a good alternative to make this meat product more attractive to the consumer, as well as having a reduced caloric intake. A 40% reduction in the product fat content and addition of FOS did not affect the color parameters or the oxidative stability. The low-fat final product had good acceptance in all the sensory attributes evaluated [62].

2.2. Incorporation of probiotic microorganisms into meat products

Probiotic microorganisms are being studied for the development of new fermented meat products that are healthier and have beneficial properties for consumers. The focus on the development of functional foods is related to the incorporation of LAB with probiotic properties in the traditional formulation. This reformulation of the traditional product aims to meet the current needs of consumers for products that have beneficial effects on human health [28].

Several microorganisms have probiotic properties, among them LAB, but some strains of non-lactic bacteria and yeasts also have beneficial effects on health. The most widely used microorganisms as probiotics in foods are Lactobacillus and Bifidobacterium [6, 21], and other genres such as Enterococcus, Saccharomyces and Pediococcus [79]. The probiotic properties of LAB are strain dependent, so each strain needs to be evaluated for its probiotic properties before being used in food processing. A combination of phenotypic and genetic techniques to accomplish the identification, classification, and typing before the use of any potential probiotic strain are recommended [23, 89]. In Brazil, for example, the National Health Surveillance Agency (ANVISA, BRAZIL) announced that the probiotic food claim must be linked to consistent scientific evidence. If a food contains more than one microorganism or product (as prebiotics), the effect on health must be demonstrated for the combination of components/ingredients, through documented clinical studies [90]. Regular intake of food containing probiotics can lead to nutritional and physiological benefits (**Table 1**) [89- 91].

Table 1. Nutritional benefits of functional foods added to probiotic microorganisms.

Nutritional benefits	Possible causes and mechanisms
Digestibility / Increase nutritional value	Partial breakdown of proteins, fats and carbohydrates. Elevate the levels of essential vitamins and amino acids
Improved use of lactose	Improve lactose digestion in individuals with lactose maldigestion
Antagonistic action against enteric pathogens	Prevention of pathogenic adhesion, production of microbial inhibitors, contributes to a normal microbiota and reduces bloating
Colonization of the intestine	Survival in gastric acid, resistance to lysozyme and low surface tension of the intestine, adherence to the mucosa, multiplication in the gastrointestinal tract Control of obesity, Lipid and glucose metabolism, control of satiety hormones
Carcinogenic effects	Inhibitory action of some types of cancer, in particular the gastrointestinal tract
Hypocholesterolemia effect	Production of inhibitors of cholesterol synthesis
Immunomodulation	Stimulation in the production of γ-interferon suppressor cells
Anti-inflammatory activity	Control of Crohn’s disease and ulcerative colitis.

Source. Adapted from Kerry et al. (2018); Champagne et al. (2018); Delgado et al. (2018) [89, 90, 91].

In order to present high viability, the probiotics should remain viable under adverse conditions in the salami production process, including fermentation, drying and ripening (addition of salt and preservatives, low pH and low water activity). Also, after consumption, the probiotic microorganisms continue to be exposed to harsh conditions, such as the passage through the gastrointestinal tract of the consumer to later exert its beneficial effect. As a result, the incorporation of LAB in fermented meat products constitutes a crucial challenge, due to the complexity of the food matrix and the probiotic sensitivity to the additives used, as well as the process conditions. LAB present rapid acidification, thus promoting the development of the sensorial characteristics of the product, as well as guaranteeing the microbiological safety against pathogens [92].

The success in the incorporation of probiotic microorganisms in fermented meat products is highly dependent on their viability during processing and product shelf-life. This viability can be affected by several technological factors such as pH, acidity, presence of other microorganisms, water activity, processing and storage temperature, concentration of additives (salt, sugar, nitrite, and nitrate) and composition of the protein matrix [93]. The study of the viability of LAB to these adverse conditions can be also a useful strategy to select probiotic strains for their use in the development of fermented meat products with probiotic potential. Indeed, some authors have already studied this [94, 95].

Despite these barriers, meat fermented sausages are considered excellent vehicles for probiotics due to the protection of the bacterial cells exerted by the fat molecules in the passage through the gastrointestinal tract [28] and the stimulation of probiotic growth by the presence of the prebiotic fibers.

The species of probiotic microorganisms most commonly used in fermented meat products are: *Lactobaccillus plantarum, Lactobaccillus casei, Lactobaccillus sakei, Lactobaccillus paracasei* and *Lactobaccillus rhamnosus* [28, 54-57, 69, 96, 97].

Lactobaccillus sp. are the most resistant bacteria to low pH values and present good adaptation in different food matrices, mainly because they are a type of naturally occurring species in fermented products [24]. Most probiotic cultures come from the healthy intestine of humans or animals, so it is recommended that the microorganisms used in the production of probiotic foods are of human origin. In the initial phase, probiotic cultures are selected as a function of not only the functional criteria but also the technological aspects and safety. The appropriate selection of probiotic strain and technological improvements in the preparation of probiotic cells directly interfere with their performance [98].

The criteria used to select these probiotic strains for use in food are related to safety, functionality and viability. Functionality aspects include tolerance to gastrointestinal tract, bile resistance, production of antimicrobial compounds and adhesion to intestinal cells. In relation to viability, the presence of viable cells in the final product is necessary in an adequate count, so that this food is considered probiotic [28, 34, 91, 99]. After the incorporation of a probiotic strain into a food, the viability of the strain needs to be monitored throughout its useful life, and the most common quality evaluation is the counting of viable cells [100]. About the recommended dose, no specific cell count is recognized to guarantee a health effect in the person, even though a dose of log 9 CFU/day is generally recommended [92]. Another author suggests that the concentration of probiotic bacteria should reach values between log 6- log 7 CFU/g product [101].

Although LAB and *Bifidobacterium sp.* are Generally Recognized as Safe (GRAS) as probiotic cultures, additional tests should be performed to check their safety, assessing their resistance to antibiotics, investigating the presence of virulent genes, production of biogenic amines and determining hemolytic activity. Nevertheless, before being used as a probiotic, the safety of the strains needs to be assessed to ensure that they will not represent a risk to consumer health. Therefore, determining whether a strain is safe is of great concern among researchers [102].

Biogenic amines are generally produced by microbial decarboxylation on specific amino acids, mainly tyrosine, histidine, phenylalanine, lysine, ornithine, and glutamate, in which the microorganisms remove the carboxyl group with the formation of the correspondent amine and CO_2. Tyramine and beta phenylethylamine are particularly abundant in food from animal origin (including fermented sausages) since these matrices are very rich in the precursor amino acids, tyrosine, and phenylalanine, used by animals to synthesize catecholamines [103].

Due to their toxicity, the biogenic amines can cause adverse effects on the consumers, such as allergies, hypertensive crises, and headaches [103, 104]. In general, there is no legal regulation about the biogenic amines content in food. This is mainly due to the individual toxicological thresholds and, also, the low levels of biogenic amines in food are not considered a serious risk to the consumer. However, they can be toxic when present in high concentrations (50 to 100 mg) [105].

Furthermore, the presence of dangerous amounts of biogenic amines is associated with a significant growth (> log 7 CFU/g) of decarboxylating microorganisms [104]. To avoid such a risk, the selection of starter cultures that present either amino oxidase activity or bacteriocin synthesis capability should be considered to prevent the undesired consequences of spontaneous fermentation [103].

2.3. ***Effect of probiotics on the technological and sensory characteristics***

Another criterion used to select a probiotic strain for the fermentation of a meat product is the effect on the technological and sensorial characteristics of the final product. In this context, it is possible to include strains that produce aroma compounds, aid in the development of color [106] or improve product safety, by the production of antimicrobial compounds. The probiotic bacteria also provide increased safety and stability to the product [12]. The sensory characteristics of probiotic foods may differ from the traditional formulation due to the addition of these probiotic strains, which can produce different metabolic compounds, such as organic acids, lipolytic and proteolytic enzymes and volatile compounds [12]. To avoid changes in sensory characteristics of the final product, the correct choice of probiotic strain is of utmost importance. This addition of the probiotic microorganism is done by replacing the traditional starter culture or by using the traditional culture in consortium with the probiotic strain [34]. In addition, there is a growing interest in increasing the viability of the strains during the useful life of the fermented product. Several reports regarding the incorporation of probiotic strains in the fermentation of meat sausages with high viable cell counts during storage are summarized in **Table 2**.

The production of a fermented pork sausage (Fuet) using *L. rhamnosus* CTC1679 showed a count close to log 8 CFU/g. This count remained stable during the 14 days of fermentation and 74 days of storage at 4 °C packaged in a modified atmosphere (80%:20% N_2/CO_2). Furthermore, *L. rhamnosus* strain CTC1679 used as a protective culture inhibited the growth of pathogenic bacteria such as *L. monocytogenes* [39, 58]. Some authors observed positive results with the use of different strains of *L. rhamnosus* GG, E-97800 and LC-70 as substitutes of commercial starter culture in fermented pork sausages in which the pH value was less than 4.9 [56, 107].

In a study using *L. acidophilus* Bauer and *L. casei* Bif3 '/ IV for the dry fermented pork salami production, the results showed a good ability of the strains to survive under the processing conditions, with the potential to replace the starter culture without altering the sensorial and technological characteristics [108].

Macedo et al. [54] studied dry fermented sausages using *Bactoferm* T-SPX (Chr-Hansen, Denmark) as starter culture. This culture contained *Staphylococcus xylosus* DD-34 and *Pediococcus pentosaceus* PC-01. Three probiotic species of *Lactobacillus* were tested separately: *L. casei* LC01, *L. paracasei* ssp. *paracasei* (ATCC 10746/CCT 0566) and *L. casei* ssp. *rhamnosus* (ATCC 7469/CCT 6645). The addition of *Lactobacillus* cultures promoted a faster pH reduction, resulting in a residual acid taste that was perceived by consumers. The probiotics presented an increase in the number of viable cells during the fermentation process (3 days). During the ripening (21 days), the probiotics count decreased but remained close to log 6 CFU/g.

Table 2. Fermented meat products and probiotic strains used

Product	Probiotic strain	Reference
Pork fermented sausage	*L. rhamnosus* FERMP-15120 *L. paracasei ssp. paracasei* FERMP-15121	Sameshima et al [109].
Fermented sausage	*L. gasseri* JCM1131T *L. fermentum* CTC1693, *L. casei/paracasei* CTC1677, CTC1678, *L. rhamnosus* CETC1679, *L. gasseri* CTC1700, CTC1704 *L. casei* CRL431	Arihara et al [110] Rubio et al [58]. Bagdatli; Kundakci [111]
Fermented sausage (*Fuet*)	*L. rhamnosus* CTC1679	Rubio et al [39].
Dry fermented sausage	*L. rhamnosus* GG *L. rhamnosus* E-97800 *L. rhamnosus* LC-705	Erkkilã et al [34].
Raw fermented sausage	*L. casei* LOCK 0900	Trzaskowska et al [60].
Italian Salami	*L. casei* *L. rhamnosus* *L. acidophilus*, *B. lactis*	Rebucci et al [112]. Ruiz et al [57].
Dry Salami	*L. fermentum HL57* *P. acidilactici SP979*	Ruiz-Moyano et al [113].
Fish-based products	LAB isolates from fish intestinal microbiota	Speranza et al. [114].
Fermented beef sausage	*L. plantarum* IIA-2C12 *L. acidophilus* IIA-2B4 *L. plantarum* TN8 and *P. acidilactici* MA18/5M	Arief et al [115]. Ben Slima et al. [61].
Mutton fermented sausage	*L. acidophilus* CCDM 476 *B. animalis* 241[a]	HOLKO et al [116].

A viable cell count of approximately log 8 CFU/g at the end of ripening (day 28) was observed in a commercial starter culture and three probiotic strains of *Lactobacillus rhamnosus* (GG, E-97800, LC-705) added in beef and pork fermented sausage [34]. The probiotic strains evaluated presented satisfactory results for dry fermented sausage production.

The addition of LAB strains (*L. plantarum* TN8 and *P. acidilactici* MA18/5M) together in the production of fermented beef sausage showed potential probiotic agents for use in the meat industry. The count of *Enterobacteriaceae* decreased in the inoculated sausages, reaching values below log 2 CFU/g. Moreover, the LAB count ranged from log 8.22 to log 8.45 CFU/g at the end of storage; these values were higher than those observed for the control samples. Besides that, the addition of probiotic strains improved the quality (improved cooking yield and texture) of beef sausage, thus these strains being a useful bioprotective tool to extend the shelf-life of sausages [61].

3. CONCLUSIONS

The modification of traditional meat products to produce functional foods can be achieved by replacing or adding of ingredients using dietary fibers and probiotic cultures that can enhance the nutritional value or provide health benefits, such as. The use of these ingredients in the fermentation of meat products is considered a promising strategy in the development of these types of products. Considering that each probiotic strain presents specific properties in relation to physiology, morphology and technological effects, it is essential to perform preliminary tests to know all these characteristics and thus to determine which is the best strain to be used for the industrial production of a specific fermented meat product. One of the next steps in area of meat product research could be the development of innovative formulations, not only with added dietary fibers and probiotics, but also combined with reduced salt, nitrite/nitrate or cholesterol content, making the development of healthier fermented meat products possible.

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CAPÍTULO 2

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Can dietary fiber improve the technological characteristics and sensory acceptance of low-fat Italian type salami?

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Abstract: To meet the needs of new consumers, meat researchers need to develop healthier products. Dietary fibers can be added for structural purposes, to present functional characteristics or to change the composition of the final product. In this study, mixture design was used to investigate the effects of partial substitution of pork fat by inulin, fructooligosaccharides and α-cyclodextrin on the technological and sensory quality characteristics of low-fat Italian type salami. The partial substitution of fat using dietary fibers shows no effect on weight loss, Aw and pH during ripening time. However, the addition of up to 2% α- cyclodextrin increased lightness and reduced redness and yellowness. Up to 2% of inulin or fructooligosaccharides added improved the sensory acceptance, texture parameters and redness. Healthier low-fat Italian type salami can be produced using inulin or fructooligosaccharides as fat substitute for pork fat and still obtain good technological and sensorial results.

Keywords: Fat substitute · Inulin · Fructooligosaccharides · α- cyclodextrin · Improved redness· Fermented sausage.

1. Introduction

In Brazil, processed meat products represent a high percentage (95%) of the consumption of pork (ABPA 2012). Fermented sausage is a well-known preparation among Brazilian consumers, and the Italian type salami can be found throughout the country. Italian type salami can contain high levels of saturated fat (up to 32%) according to the physico-chemical parameters (Brazil 2000) and can have high salt content, a lack of bioactive molecules and poor fiber content (Pérez-Burillo et al. 2019). Changes in consumer behavior have steadily increased the trend towards healthier food products (Hilbig et al. 2019), and this consequently compromises the consumers' interest in traditional products (Bis-Souza et al. 2019).

The reduction in the fat content of a meat product usually implies undesirable effects to technological, structural and sensory characteristics (Henning et al. 2016), such as a reduction in yield, negative changes in texture and a reduction in sensorial acceptance (Han and Bertram 2017). The use of dietary fiber offers a healthier meat product with higher fiber content. It is, also an alternative ingredient that can maintain the technological and sensory properties (Henning et al. 2016; Zhao et al. 2018). Additionally, consumer preference for meat products that provide benefits to physiological functions and have greater nutritional value has increased significantly (Han and Bertram 2017). Inulin, fructooligosaccharides and α-cyclodextrin are examples of oligosaccharides extracted from plants that have an important gelling property (Bis-Souza et al. 2019). The addition of this dietary fiber in fermented meat products can improve the content of short chain fatty acids (SCFAs), which represents a component associated with some health benefits (Pérez-Burillo et al. 2019).

Moreover, most of these fibers have a neutral flavor, which minimizes changes in the sensory acceptance of the meat product (Santos et al. 2013; Díaz-Vela et al. 2017; Marino et al. 2017; Glisic et al. 2019). Accumulated data suggests that a high-prebiotic fiber diet presents many health benefits, such as a decrease in the relative risks of developing obesity, coronary artery disease and diabetes (Casarotti et al. 2018). Prebiotic fibers, such as inulin and oligofructose, increase the concentration of acetate and propionate, improve gut-barrier function, induce weight-loss and reduce food intake (Bailey and Holscher 2018).

The use of dietary fiber, such as inulin, arabinogalactan, citrus fiber, fructooligosaccharides, oligofructose, lactulose, galactooligosaccharides, alone or in combination, has already been evaluated as a fat substitute in meat products. The efficiency of such fiber has already been proved in emulsified meat (Sarteshnizi et al. 2015; Alves et al. 2016), fermented sausages (Park et al. 2017; Glisic et al., 2019; Pérez-Burillo et al. 2019), cooked products (Keenan et al. 2014; Henning et al. 2016; Díaz-vela et al. 2017, Han and Bertram 2017) and restructured meat (Bis-Souza et al. 2018). For example, the partial substitution of pork back fat by inulin gelled suspension decreased hardness and chewiness and showed no effect on the acceptability of the sensory attributes of fermented sausage (Glisic et al., 2019). Finally, a recent study with consumers found that the addition of dietary fiber in meat products can change the consumers' perception about those products, turning frankfurters with dietary fiber and omega-3 added into a potential marketable product (Rocha et al. 2019).

The Simplex-centroid mixture design allows the study for the best condition of three different ingredients for a given response-variable and it is also possible to optimize the experiment (Anarjan et al. 2017; De Moraes Filho et al. 2018). We did not find references in the literature about the effect of a mixture of three types of prebiotic dietary fibers added in the same salami formulation. Thus, the aim of this study was to evaluate the effect of the partial substitution of pork fat by combinations of prebiotic dietary fiber (inulin, fructooligosaccharides and α-cyclodextrin) in Italian type salami on sensory and technological characteristics using the Simplex-centroid mixture design.

2. Materials and methods

2.1. Ingredients

The dietary fibers used in this study were: Clariant - Orafti® inulin (moisture 3 $g.100g^{-1}$ of moisture; total dietary fiber 90 $g.100g^{-1}$) (Beneo, Mannheim, Germany); Ingredion® - fructooligosaccharide (FOS) (4 $g.100g^{-1}$ of moisture; total dietary fiber 95 $g.100g^{-1}$ (Ingredion, Westchester, USA) and from the α-cyclodextrin (ACD) (Vogler Ingredients, São Bernardo do Campo, Brazil). The fresh pork meat and the pork fat were purchased from a local market (Carnes Pompéia, Campinas, Brazil).

2.2. Experimental design – simplex-centroid mixture design

The three dietary fibers: inulin – (X_1), fructooligosaccharide (FOS) - (X_2), α-cyclodextrin (ACD) – (X_3), were used in the mixture design. The hypothesis was tested by a matrix composed of 10 mixtures (7 different assays and 3 replicates with 1 type of dietary fiber), 6 assays with only 1 type of dietary fiber, 3 assays were the binary mixtures of two ingredients and 1 assay was the ternary mixture of three ingredients (**Table 1**). The maximum amount of dietary fiber added was 2%. The amount of fiber added in the formulation was defined based on results from the literature and on the claim related to the fiber content prevailing in Brazilian legislation (Brazil 2012). A product can claim to be a "source of fiber" if it has a minimum concentration of 2.5 g of fiber per portion or 3 g of fiber per 100g of product. Therefore, it was decided to add 2 g.100g^{-1} of the dietary fiber, considering an average yield of 60% of the low-fat Italian type salami.

The Simplex-centroid mixture design was used to evaluate in which proportion the added fibres presented the best results for the sensorial and technological characteristics studied. And so, it is possible to select the best formulation for fat reduction in Italian type salami.

The response-variables were moisture, fat content, weight loss, lightness, redness, yellowness, hardness, cohesiveness, springiness, chewiness and the 2-thiobarbituric reactive substances (TBARS) value.

2.3. Low-fat Italian type salami formulation and preparation

Ten formulations of low-fat Italian type salami were produced with 80 g.100g^{-1} of fresh pork shoulder (Landrace, moisture 74 g.100g^{-1}, fat 6 g.100g^{-1}) and 10 g.100g^{-1} of pork back fat (moisture 11 g.100g^{-1}, fat 82 g.100g^{-1}) obtained from a slaughterhouse with federal inspection service (FIS). The others ingredients were: sodium chloride (2.5 g.100g^{-1}), sucrose (2 g.100g^{-1}), sodium nitrite (0.015 g.100g^{-1}), sodium nitrate (0.015 g.100g^{-1}), garlic (0.2 g.100g^{-1}), white pepper (0.5 g.100g^{-1}), nutmeg (0.2 g.100g^{-1}), sodium erythorbate (0.3 g.100g^{-1}), and a starter culture (0.025 g.100g^{-1}; Bactoferm T-SPX (Chr. Hansen, Hoersholm, Denmark) composed of *Pediococcus pentosaceus* and *Staphylococcus xylosus*. The dietary fiber was added according to **Table 1**.

Table 1. Simplex-centroid mixture design matrix to low-fat Italian type salami.

	Matrix			Proportion (%)		
Treatments	X_1	X_2	X_3	Inulin	FOS	ACD
I1	1	0	0	2	0	0
F1	0	1	0	0	2	0
A1	0	0	1	0	0	2
I2	1	0	0	2	0	0
F2	0	1	0	0	2	0
A2	0	0	1	0	0	2
IF	0.5	0.5	0	1	1	0
IA	0.5	0	0.5	1	0	1
FA	0	0.5	0.5	0	1	1
IFA	0.33	0.33	0.34	0.66	0.66	0.68

I1; I2 (10% of pork fat + 2% of Inulin); **F1; F2** (10% of pork fat + 2% of FOS); **A1; A2** (10% of pork fat + 2% of ACD); **IF** (10% of pork fat + 1% Inulin + 1% FOS); **IA** (10% of pork fat + 1% Inulin + 1% ACD); **FA** (10% of pork fat + 1% FOS + 1% ACD); **IFA** (10% of pork fat + 0.66% Inulin + 0.66% FOS + 0.68 ACD).

The samples were produced in the Laboratory of Meat and Derivatives at the Faculty of Food Engineering, University of Campinas (São Paulo, Brazil). All the ingredients were mixed using an automatic blender for approximately 5 minutes. After, the samples were stuffed in cellulose cases (diameter of 50 mm) and separated into pieces of 15 cm, totaling 25 pieces (approximately 250 g) for each treatment. Afterwards, samples were soaked in a solution of potassium sorbate (20 $g.100g^{-1}$) and ripened for13 days. The temperature and relative humidity parameters (T ºC and RH %) were controlled over the ripening time, 15ºC-25ºC and 75%-95%, respectively. The air velocity was also controlled throughout the processing (0.5 $m.s^{-1}$).

2.4. pH value and water activity evaluations during ripening time

The pH value was measured with a penetration probe in a PG 1800 digital pH meter (Gehaka, São Paulo, Brazil). Water activity (Aw) was measured using an Aqualab water activity meter (Decagon Devices Inc., Pullman, USA). The analyses were performed during ripening time on days 0, 3, 7, 10 and 13 after the stuffing process. The analyses were performed three times on each of the five salami sampled for each ripening time.

2.5. Weight loss and general composition

Five sausages (with an initial weight of approximately 250 g) from each treatment were initially weighed. The same samples were reweighed on days 3, 7, 10 and 13. Weight losses were expressed as the percentage of the initial weight (Glisic et al. 2019). All the treatments were characterized for moisture content according to Horwitz and Latimer (2007) and the fat content according to Bligh and Dyer (1959). These analyses were performed on day 0 and after the full ripening time (day 13). All analyses were performed in triplicate.

2.6. Lipid oxidation analysis (TBARS)

The degree of lipid oxidation was evaluated by measuring the TBARS value, according to Vyncke (1970) with modifications. The TBARS value was measured from a standard curve of malonaldehyde (MA) and expressed as mg $MA.kg^{-1}$ of sample. The analyses were performed in triplicate.

2.7. Characterization of color parameters

The instrumental color was measured in a ColorFlex 45/0 spectrophotometer (Hunterlab, Virginia, USA), observation angle of 10 º, illuminant D65 and linked to Universal software version 4.10 (Hunterlab, Virginia, USA). The specification system used was the CIELAB color space expressing the three values: L* for the lightness from black to white, a* from green to red, and b* from blue to yellow. Ten readings were performed per sample (n=5). The analyses were performed after the ripening process. The samples were left at room temperature for 30 minutes before analysis.

2.8. Texture profile analysis (TPA)

The instrumental texture profile analysis was performed at room temperature (25 ºC) using a TA-XT/Plus/50 texture analyzer (Stable Micro Systems, Godalming, UK), equipped with a cylindrical probe of 36 mm in diameter. The parameters hardness (N), cohesiveness, springiness (mm) and chewiness ($N.mm^{-1}$) were determined, as described by Bourne (1978) and Szczesniak (2002). The samples of low-fat Italian type salami were cut into slices 1.5 cm thick and were axially compressed 50% of height (0.75 cm) in two consecutive cycles, at a constant speed test of 1 $mm.s^{-1}$ (n=10).

2.9. Microbiological analysis

After the ripening process, the analyses determined the count of the thermo-tolerant coliforms, the presence of coagulase-positive *Staphylococci* and *Salmonella sp*. in 25 g was investigated according to the Brazilian Health Surveillance Agency (Brazil 2001) and the results were expressed in log $CFU.g^{-1}$ of sample.

2.10. Sensory acceptance

A consumer study was conducted by 89 untrained panelists recruited among students and staff members from São Paulo State University (UNESP) at the São José do Rio Preto campus (SP, Brazil). These panelists were previously selected by a questionnaire related to the frequency of consumption of fermented meat products. All the panelist that agreed to participate in the sensory analysis had to declare their consent in a pre-filled form before the analysis began. The sensorial attributes evaluated in the acceptance test were appearance, color, taste, texture and overall acceptance using a structured hedonic scale of 9 points. Sensory evaluation was carried out only for the different formulations of low-fat Italian type salami (n=7), after the ripening process. Each sample was coded with a three-digit number and the presentation was randomized in a sequential monadic way, following a balanced complete block design (Meilgaard, Civille & Carr, 1999). The tests were performed in two sessions, in individual booths under white light and at a temperature of 22 ºC. All panelists evaluated one sample of all treatments in a randomized order. This project was approved by the Ethics in Research Committee of São Paulo State University (São José do Rio Preto, Brazil) under protocol number 2.126.575.

2.11. Statistical analysis

The results were expressed as the mean values and the standard error of the mean (SEM). Differences in pH and water activity (Aw) were analyzed statistically using mixed model ANOVA analyses and the means were compared using the Tukey HSD test ($P < 0.05$). Each triplicate of each batch was included as a random term and different times were included as fixed term.

To analyze the data of the response-variables: moisture, fat content, weight loss, lightness, redness, yellowness, hardness, cohesiveness, springiness, chewiness and TBARS value, the linear and quadratic models for the mixture design were used. Mixture analysis was used to determine the effects, the linear, quadratic, and cubic regression coefficients, the lack of fit and the coefficient of determination (R^2) for each response-variables. The quadratic model showed better fit to the data, so the results were presented following Equation 1.

$$y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

For the sensory analysis the treatment was considered as the main effect and consumers as random variable. The data obtained in the sensorial analysis were analyzed statistically using the General Linear Model (GLM) and the means were compared using the Tukey test ($P < 0.05$). The results were also expressed as the mean values and the standard error of the mean.

All experimental design, data analysis, regression and optimization steps were completed using Statistica 7.0 software (Statsoft Inc., USA).

3. Results and discussion

3.1. pH value and water activity during ripening time

During the processing of fermented meat sausage, monitoring two technological parameters is extremely important to achieve product stability: (i) the decreasing of the pH which depends exclusively on the acidification caused by lactic acid bacteria and (ii) water activity that is related to the product water loss to the environment during drying process (Prado et al., 2019). There were no significant (P>0.05) differences between treatments of low-fat Italian type salami during the ripening time for water activity (Fig 1.a) and pH (Fig 1.b). The reduction in Aw and pH during the ripening process guarantee a safe and microbiologically stable product (Ferreira et al., 2006; Prado et al., 2019).

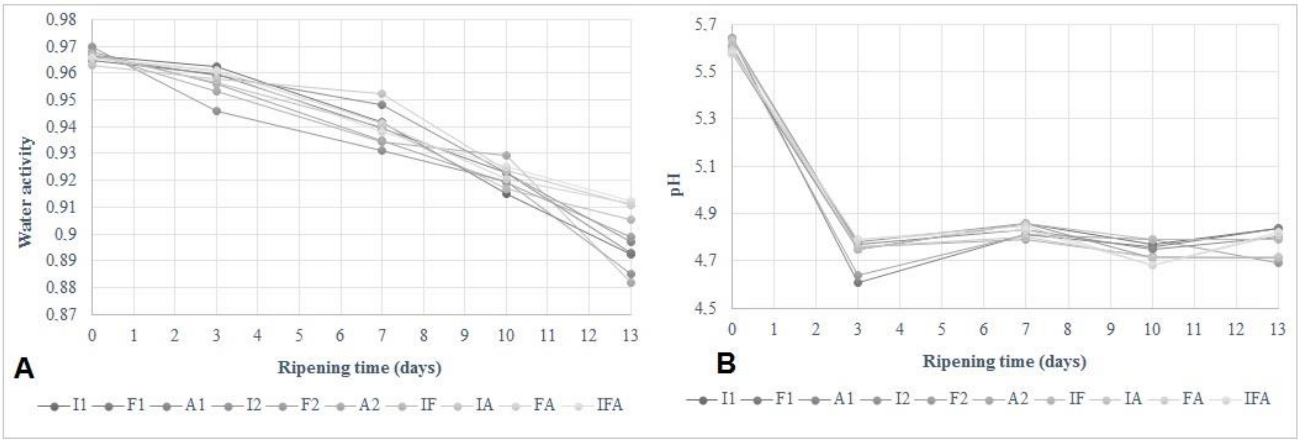

Figure 1. Water activity **(a)**, pH value **(b)** of low-fat salami type Italian during ripening time. Not significantly results (P>0.05) for both evaluations. **I1; I2** (10% of pork fat + 2% of Inulin); **F1; F2** (10% of pork fat + 2% of FOS); **A1; A2** (10% of pork fat + 2% of ACD); **IF** (10% of pork fat + 1% Inulin + 1% FOS); **IA** (10% of pork fat + 1% Inulin + 1% ACD); **FA** (10% of pork fat + 1% FOS + 1% ACD); **IFA** (10% of pork fat + 0.66% Inulin + 0.66% FOS + 0.68 ACD).

The initial value of Aw for the batches were between 0.96 – 0.97. During ripening processes of dry fermented sausage, the product loses water because of evaporation (lower relative humidity in the maturation chamber), which causes the Aw to decrease. However, the addition of dietary fiber in low-fat Italian type salami had no effect (P>0.05) on Aw. Similar results were found when amorphous cellulose gel (Campagnol et al. 2012) and a mixture of collagen and dietary fiber (Ham et al. 2016) were used as fat substitutes in low- fat fermented sausages.

The initial pH was around 5.6 for all the low-fat Italian type salami batches. After 3 days (fermentation), the pH value decreased to 4.6. This was observed for all low-fat salami. The use of inulin and corn oil as fat substitute in dry-fermented chicken sausage (Menegas et al. 2013) or orange fiber as fat substitute in Spanish "*Salchichón*" (Fernández-lópez et al. 2008) or FOS in dry fermented sausage (Salazar et al. 2009) or linseed oil gelled emulsion as pork back fat substitute in a dry fermented sausage (Alejandre et al. 2016) or with a fat content reduction in fermented sausage (Yim et al. 2016) no differences in the pH were reported.

3.2. Weight loss and general composition

The addition of dietary fiber as fat substitute in low-fat Italian type salami showed (*P*>0.05) no effect on the weight loss (**Table 2**).

Table 2. Experimental results of moisture, fat and weight loss for low-fat Italian salami before and after the ripening time.

Treatments	Initial		Final		
	Moisture	Fat	Moisture	Fat	Weight loss (%)
I1	66.01	9.72	36.22^{b}	22.85^{ab}	40.39
F1	66.24	9.60	39.43^{a}	22.44^{ab}	40.52
A1	66.97	9.07	36.86^{b}	21.08^{ab}	39.89
I2	66.21	10.51	36.94^{b}	23.39^{a}	40.54
F2	66.47	9.93	38.95^{a}	21.95^{ab}	41.05
A2	65.55	10.23	35.98^{b}	20.75^{b}	40.50
IF	67.66	9.52	35.99^{b}	21.26^{ab}	39.57
IA	66.13	10.23	36.51^{b}	22.09^{ab}	39.73
FA	66.67	9.21	36.62^{b}	22.46^{ab}	40.68
IFA	66.84	9.17	39.70^{a}	22.15^{ab}	40.49
SEM	0.16	0.13	0.32	0.21	0.20
p value	*0.131^{ns}*	*0.112^{ns}*	*<0.0001*	*0.036*	*0.914^{ns}*

[a a,b]According to the Tukey test, samples on the same column with different letters show present significant differences between them (P < 0.05). SEM: Standard error of mean [ns] not significant. **I1; I2** (10% of pork fat + 2% of Inulin); **F1; F2** (10% of pork fat + 2% of FOS); **A1; A2** (10% of pork fat + 2% of ACD); **IF** (10% of pork fat + 1% Inulin + 1% FOS); **IA** (10% of pork fat + 1% Inulin + 1% ACD); **FA** (10% of pork fat + 1% FOS + 1% ACD); **IFA** (10% of pork fat + 0.66% Inulin + 0.66% FOS + 0.68 ACD).

The weight loss of a fermented sausage is related to the yield of the final product. Therefore, partial substitution of fat by dietary fibers without the need to add another ingredient to increase the yield is a technological advantage. The average weight loss of low-fat Italian type salami was 40%. Ruiz et al. (2014), Blaiotta et al. (2018) and Cruxen et al. (2018) also reported a similar weight loss during the process of fermented sausages. This technological characteristic depends on different factors, such as the temperature and relative humidity used in the processing, the air velocity inside the maturation chamber, the maturation time, the material of the wrapper and the amount of fat added in the batch (Yim et al. 2016).

There was no effect (P>0.05) of the addition of dietary fibers on the moisture and fat results of the batch (initial) for all treatments, since all formulations were made with the same amount of raw materials, the only difference being the dietary fibers added. After the processing, the highest (P > 0.05) moisture content was observed in the samples with 2% FOS (F1 and F2) and in the treatment with the 3 dietary fibers (IFA). In similar studies, the treatments with reduction in fat content and addition of amorphous cellulose gel (Campagnol et al. 2012) and inulin with corn oil (Menegas et al. 2013) as fat substitute show higher values of moisture in relation to the control formulation. The low-fat Italian type salami treatments presented different values (P<0.05) of fat content at the end of the ripening time (Table 2).

Additionally, the substitution of pork fat in fermented sausages with non-fat ingredients helps to reduce the total caloric value and cholesterol levels of the product. It could improve the viability of developing of healthier meat products from a nutritional point of view (Ham et al. 2016).

3.3. Lipid oxidation analysis (TBARS value)

The amount of malonaldehyde formed during the oxidation process of each low-fat Italian type salami formulation is quantified based on the thiobarbituric acid reactive substances (TBARS). The inulin, FOS and ACD added in low-fat salami show a positive effect *(P* < 0.05) on the lipid oxidation, increasing the TBARS value (**Table 3**). The coefficients of binary and ternary mixtures of components were not significant (P >0.05). That is, only the addition of 2% of each dietary fiber alone had a significant effect on lipid oxidation. Similar results were reported by Ham et al. (2016), Yim et al. (2016), Bis-Souza et al. (2018), when using dietary fibers as fat substitute in low-fat meat product. The highest value for TBARS was observed with addition of 2% FOS. (Fig.2.A). The addition of dietary fiber increases the carbohydrate content of the product and alters the food matrix, causing an increase in lipid oxidation. Glisic et al. (2019) suggest that the addition of inulin gelled suspension in dry fermented sausage increases the polyunsaturated fatty acids (PUFA) content, compounds highly susceptible to oxidation, thus affecting the TBARS value.

Despite these increases in the TBARS values through the addition of dietary fibers, all formulations were below 2.0 mg MDA/kg, which is accepted as the deterioration level (Greene & Cumuze, 1982).

Table 3. Regression models to experimental data in mixtures design for technological properties, textural parameters and color parameters of low-fat Italian salami.

Response	Equation[1]	Model	R^2	*p value*	Lack of fit
TBARS value	$0.35X_1 + 0.53X_2 + 0.25X_3$	Linear	0.93	0.019*	0.408
Lightness	$45.34X_1 + 40.35X_2 + 53.28X_3 + 24.64X_2X_3$	Quadratic	0.95	0.009*	0.529
Redness	$12.21X_1 + 12.61X_2 + 10.12X_3$	Linear	0.94	0.014*	0.664
Yellowness	$9.13X_1 + 8.57X_2 + 11.19X_3$	Linear	0.90	0.041*	0.838
Cohesiveness	$0.48X_1 + 0.48X_2 + 0.49X_3 + 0.12X_1X_2 - 0.12X_2X_3$	Quadratic	0.92	0.027*	0.956
Chewiness ($N.mm^{-1}$)	$16.75X_1 + 17.75X_2 + 29.47X_3 - 34.14X_2X_3$	Quadratic	0.94	0.014*	0.084

[1]X_1: Inulin, X_2: FOS, X_3: ACD. ***Significant (*P*<0.05) of regression coefficient according to ANOVA.

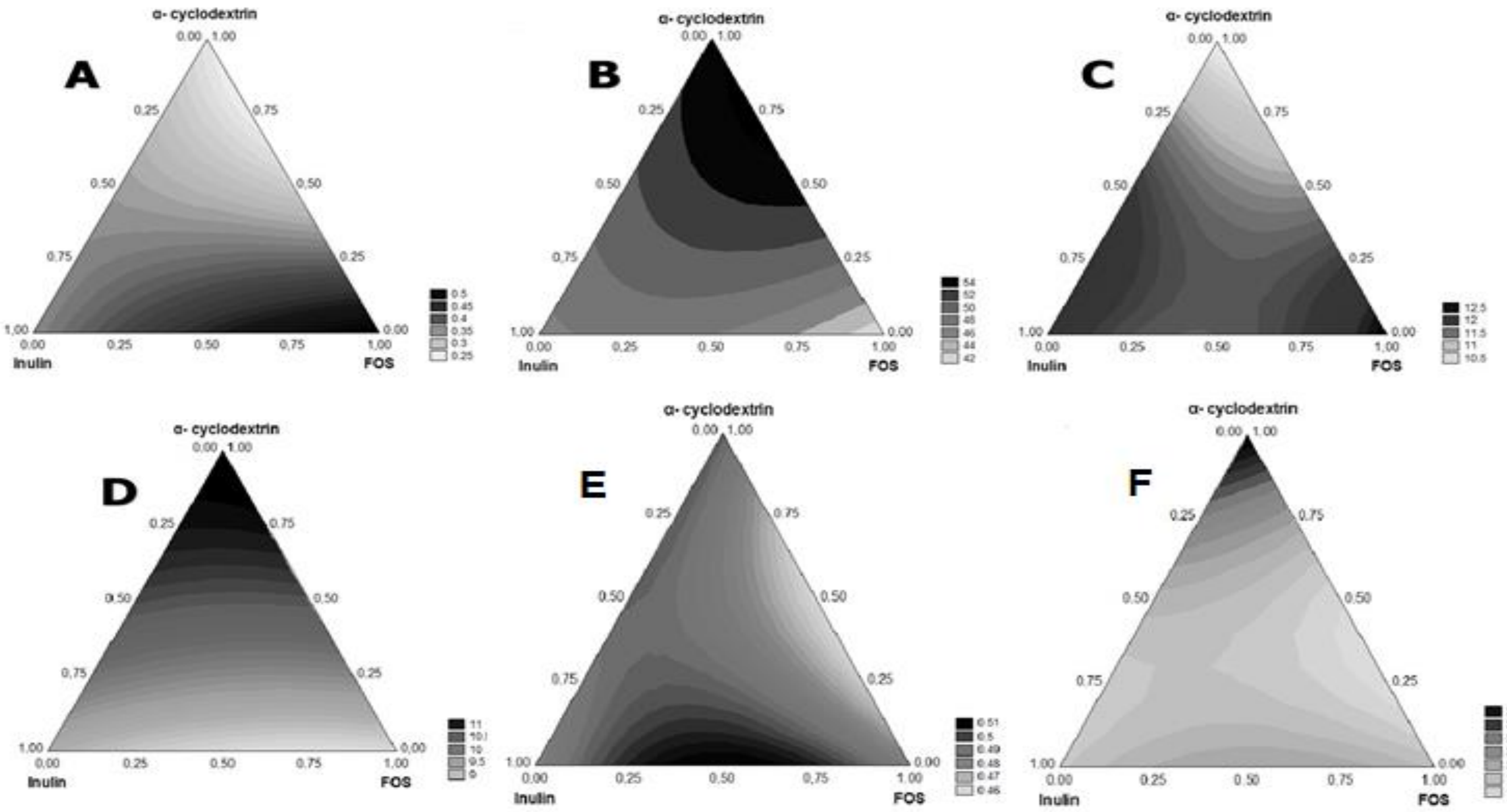

Figure 2. Contour plots of lipid oxidation (**A**) (mg MDA/kg), Lightness **(B)**, Redness **(C)**, Yellowness **(D),** Cohesiveness **(E)** and Chewiness (N.mm^{-1}) **(F)** showing the effect of dietary fiber added as fat substitute in low-fat Italian salami.

3.4. Characterization of color parameters

The addition of fiber in low-fat Italian type salami as partial fat substitutes directly and significantly ($P < 0.05$) influences the lightness of the final product. The three components when added alone increase the value of the lightness, according to the regression models (**Table 3**). A positive effect of the interaction between FOS and ACD on the increase of lightness is also seen (**Table 3**). When up to 2% of ACD was added, the lightness increased to 54 (Fig. 2.B). The addition of inulin, FOS and ACD up to 2% decreased the lightness of low-fat Italian type salami, which may justify its use in this type of product. The increased lightness of the fermented meat product may be related to the white color of dietary fibers.

Other authors have also reported an effect on lightness when adding soluble dietary fiber as a substitute for fat in meat products. This effect was reported as increasing (Caceres et al. 2004; Menegas et al. 2013) and decreasing lightness of low-fat meat products (Salazar et al. 2009; Keenan et al. 2014).

Fructooligosaccharide and inulin added to low-fat beef burger (3 and 6%) caused a reduction in lightness (Bis-Souza et al. 2018) and the addition of albedo-fiber powder from yellow passion fruit in pork burger showed no effect on the lightness (López-Vargas et al. 2014).

The use of inulin, FOS and ACD as fat substitute in low-fat Italian salami produced a significant increase ($P < 0.05$) in redness (**Table 3**, Fig. 2.C) changing from 10.5 to 12.5, when 2% of each fiber was added. Similar results were reported when 20% of fat was substituted by regenerated cellulose fiber in emulsified sausage (Zhao et al. 2018). The use of green banana flour as fat substitute in bologna type sausage also increased the redness compared to a control (Alves et al. 2016). In contrast, the use of whole buckwheat flour as pork fat substitute in salami sausage did not affect the redness (Park et al. 2017).

The three components added separately as fat substitute in low-fat salami increased the yellowness ($P < 0.05$) (**Table 3**). The study conducted by Zhuang et al. (2016) also reported a significant increase in yellowness when sugarcane dietary fiber was used as fat substitute in low-fat meat batter.

The addition of inulin and FOS decreased the yellowness compared to the ACD, which ranged from 9 to 11 (Fig 2.D). A similar finding was reported by Keenan et al. (2014), who associated the reduction in the yellowness to the increase in the inulin concentration added in the formulation of low-fat pork sausage.

3.5. Texture profile analysis (TPA)

Regarding the texture profile analysis, only cohesiveness and chewiness were significantly affected by the addition of the dietary fibers ($P < 0.05$) and these parameters could be fitted to the proposed model (**Table 3**). The hardness and springiness of low-fat salami were not affected by the addition of dietary fiber as pork fat substitute. Ham and Bertram (2017) also reported that the partial substitution of fat for different fibers showed no significant effect on springiness.

On the other hand, some authors argue that the use of a short chain soluble dietary fiber such as inulin, FOS or ACD may lead to increased hardness of meat products, since the gel formed by the fiber is more compact than the protein matrix (Caceres et al. 2004; Lopez-Lopez et al. 2010). The use of resistant starch also shows an increased hardness in prebiotic cooked sausage (Sarteshnizi et al., 2015).

The addition of the single components (inulin, FOS or ACD) and the binary mixture of inulin and FOS result in increased cohesiveness (**Table 3**). However, the addition of FOS + ACD decreased this texture parameter. The highest value of cohesiveness (Fig. 2.E) was observed when 1% inulin + 1% FOS were used, resulting in a more compact fermented meat product than when only ACD was used.

Only the use of the binary mixture of FOS+ ACD decreased the chewiness (**Table 3**). ACD promoted an increase in chewiness values, as observed in Figure 2.F. Similar results were reported by Glisic et al. (2019) when they replaced pork back fat with inulin gel in dry fermented sausage.

The partial substitution of fat in salami using whole buckwheat flour showed no influence in hardness and chewiness; however, this fat substitute contributed to decrease springiness and cohesiveness (Park et al. 2017). The differences in texture profile parameters in low-fat Italian type salami could be explained by the higher moisture content and moisture/protein ratios (Glisic et al. 2019). And, consequently, the water loss during ripening time also affects the texture parameters of the product (Zhou et al. 2018).

3.6. Microbiological analysis

The absence of *Salmonella* spp. in 25 g of sample was confirmed at the end of the test. Thermo tolerant coliforms and coagulase-positive *staphylococci* showed final counts lower than 1 log $CFU.g^{-1}$. All the results were in accordance to the Brazilian Legislation (Brazil, 2001). These results can be attributed to the low pH value and high amount of lactic acid bacteria present in the product, which are conservation factors for fermented sausages and promote the safety of the product. The good quality of raw material and good manufacturing practices used in the process also could contribute to those results.

3.7. Sensory acceptance

The lowest ($P < 0.05$) score for appearance (**Table 4**) was for the sample with the addition of 2% of ACD. In contrast, the addition of 2% inulin or 2% FOS or 1% inulin + 1% FOS or 0.66% inulin + 0.66% FOS+ 0.68% ACD resulted in a higher score for appearance ($P < 0.05$) of low-fat salami. The use of ACD (up to 2%) seems to have negative effect (6.31) on the color sensory acceptance of low-fat Italian type salami, compared to inulin or FOS added up to 2% (7.66 and 7.72, respectively). Possibly because of the reduction in redness due to the white coloration of the fibers added.

The addition of dietary fibers as fat substitute in low-fat Italian type salami showed no effect ($P > 0.05$) on flavor, regardless of the concentration (up to 2%) and the type of dietary fiber (inulin, FOS or ACD). Similar findings were presented by Zhao et al. (2018) when regenerated cellulose (1.8%) was used as fat substitute in low-fat emulsified sausage; the flavor score showed no difference to the control.

The addition of 2% ACD increased hardness and showed the lowest score ($P < 0.05$) for sensorial texture (**Table 4**). Grigelmo-Miguel et al. (1999) also observed a significant negative correlation between hardness and acceptability of low-fat high fiber frankfurter, meaning that the consumers preferred the softer products. The sensorial texture also affected the global acceptance results (**Table 4**), in which sample A (2% ACD) showed the lowest ($P < 0.05$) score for this attribute.

The results for global acceptance are in line with the previous sensorial attribute scores. The treatment with addition of 2% ACD (treatment A) showed the lowest global acceptance score (P < 0.05). The tertiary mixture of dietary fiber (treatment IFA) showed positive results for the sensory attributes of appearance, color and texture, and also for overall acceptance.

The addition of up to 2% inulin and 2% FOS by themselves or the use of 1% inulin and 1% FOS appears to have a positive effect on the sensory acceptance of low-fat Italian type salami; the treatments I, F and IF showed the highest (P < 0.05) score for appearance, color, texture and global acceptance. The ability of FOS to maintain the sensorial characteristics of the low-fat cooked sausage when used as a fat substitute had already been reported by Campagnol et al. (2012). The use of inulin as partial oil replacement in dry chicken sausages showed no significant effect in sensory characteristics during storage at 4 ° C for 45 days (Menegas et al. 2013).

Table 4. Effect of addition of dietary fiber in sensory acceptance of the different formulations of low-fat salami type Italian.

Treatment	Appearance	Color	Flavor	Texture	Global acceptance
I	7.78^{a}	7.66^{a}	7.35	7.58^{a}	7.52^{a}
F	7.64^{ab}	7.72^{a}	7.52	7.60^{a}	7.48^{a}
A	6.15^{d}	6.31^{c}	7.19	6.92^{b}	6.79^{b}
IF	7.37^{ab}	7.65^{a}	7.25	7.38^{ab}	7.40^{ab}
IA	6.82^{c}	6.79^{bc}	7.51	7.26^{ab}	7.21^{ab}
FA	7.13^{bc}	7.25^{ab}	7.22	7.18^{ab}	7.08^{ab}
IFA	7.54^{ab}	7.57^{a}	7.35	7.38^{ab}	7.43^{a}
SEM	0.060	0.056	0.055	0.056	0.053
p value	0.0023	0.0095	0.9288^{ns}	0.0179	0.0019

[a,b,c,]According to the Tukey test, samples on the same column with different letters show present significant differences between them (P < 0.05). SEM: Standard error of mean [ns]. not significant (P > 0.05). **I** (10% of pork fat + 2% of Inulin); **F** (10% of pork fat + 2% of FOS); **A** (10% of pork fat + 2% of ACD); **IF** (10% of pork fat + 1% Inulin + 1% FOS); **IA** (10% of pork fat + 1% Inulin + 1% ACD); **FA** (10% of pork fat + 1% FOS + 1% ACD); **IFA** (10% of pork fat + 0.66% Inulin + 0.66% FOS + 0.68 ACD).

4. Conclusion

Short-chain dietary fibers can be effectively used as a pork fat substitute to develop a healthier fermented meat product. The mixture design considering those dietary fibers carried out in this study showed a positive effect on the texture and color parameters in low-fat Italian type salami while the ripening parameters were not affected. Additionally, the sensory acceptance of the low-fat Italian type salami improved when up to 2% inulin or FOS were used. Therefore, the partial substitution of pork fat by inulin or FOS is a potential strategy for the meat industry to support the fermented product a functional claim related to the benefits of dietary fiber intake.

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CAPÍTULO 3

Artigo submetido ao periódico *Meat Science*.

Applicability of potentially probiotic *Lactobacillus casei* in low-fat Italian type salami added with fructooligosaccharides: screening *in vitro* and technological evaluation.

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Abstract

The aim was to evaluate the applicability of *Lactobacillus casei* strains in the fermentation process of low-fat Italian type salami with fructooligosaccharides (FOS). A screening using four probiotic strains were performed in under pH 5.5, 5.0 and 4.5 and incubation temperatures (15 and 25 ºC) at different time. *Lactobacillus casei* SJRP66 and *Lactobacillus casei* SJRP169 were selected and applied in low-fat fermented sausage - C, FOS (25% fat reduced with 2% FOS), FOS_66 (25% fat reduced with 2% FOS and *L.casei* SJRP 66) and FOS_169 (25%f at reduced with 2% FOS and *L.casei* SJRP 169). The evaluation included pH, moisture, lactic acid bacteria count, probiotic count and weight loss during ripening time, instrumental color, TBARS and texture parameters. FOS_66 and FOS_169 presented a good probiotic count (8 log CFU/g), similar technological behavior than C treatment. The probiotic addition shows no effect in the lipid oxidation and a* value. This strains probiotic showed promising properties for applications in low-fat Italian type salami with healthier appeal.

Keywords: Fermented sausage, reformulation, LAB count, probiotic strains.

1. Introduction

In the last years, the consumer preference for healthier and safer food products is growing (Coelho et al., 2019), and this change in consumption has been imposing rules in the area of food technology, both scientific and industrial. The use of probiotic strains in the processing of fermented meat products have won space because of the numerous benefits provided to the consumer by the intake of these microorganisms (Bis-Souza et al., 2019a).

The administration of probiotics strains can improve the well-being and the gut health in humans and animals (Salotti de Souza et al., 2019). In the case of these benefits, it can be highlighting the improving in several medical conditions like lactose intolerance, cancer, allergies, hepatic diseases, reduction of total cholesterol and increasing in the resistance to infection by the affect in the immune system (Bergillos-Meca et al., 2015; Casarotti et al., 2017; Salotti de Souza et al., 2019). The ability of the LAB strains to compete with the natural microbiota of the raw material and to undertake the metabolic activities expected is conditioned by its growth rate and survival in the conditions prevailing in the Italian type salami i.e., an anaerobic atmosphere, rather high salt concentrations, low temperatures and low pH value.

LAB can use various mechanisms to overcome the damage in response to stress by acid, including maintaining the intracellular pH and cell membrane functionality and inducing stress-response proteins (Wu, He, & Zhang., 2014). Those mechanisms vary by species and according to exogenous conditions, including the growth media and incubation conditions (Madureira et al., 2011).

Beyond the health benefits, fermented meat products present favorable conditions for carrying probiotic microorganisms as a function of lipid protection in bacterial cells during passage through the gastrointestinal tract (Rouhi, Sohrabvandi, & Mortazavian, 2013; Libera, Karwowska, Stasiak, & Dolatowski., 2015; Pavli et al., 2020). In addition, the fermented sausages are ready for consumption, also meeting the demand for practicality and convenience. Another demand that is more related to the trend towards healthier and more functional products is the low-fat meat products (Gómez & Lorenzo, 2013; Alves et al., 2016; Heck et al., 2017). A widely used reformulation strategy that has shown satisfactory technological results to alleviate the effects of reduced fat content on meat products is the addition of prebiotic fibers such as FOS and inulin (Pérez-Burillo et al., 2019; Glisic et al., 2019; Bis-Souza et al., 2020).

The satisfactory application of these microorganisms in fermented meat products should ensure the survival of these cells throughout the fermentation and drying process of the product under different temperatures, in addition to the limiting conditions presented by the matrix (Rouhi et al., 2013; Cavalheiro et al., 2015; Fenster et al., 2019). So, it is necessary to carry out an initial *screening* to select strains with industrial aptitude, which include, high cell viability in the meat matrix and during processing conditions.

The possible impact into the safety and health benefits to the consumers of fermented meat products has a positive influence in the search for new strains with probiotic potential with applicability in this type of food product (Aarti and Khusro, 2019; Trabelsi et al., 2019; Pavli et al., 2020). Thus, the purpose of this study was to evaluate the applicability of potentially probiotic strains of *Lactobacillus casei* on the manufacturing of a low-fat Italian type salami.

2. Material and methods

2.1. Probiotic cultures of Lactobacillus casei

The potentially probiotic strains of LAB used in this study were from the Culture Collection of Lactic Acid Bacteria of São Paulo State University, UNESP (CCLAB-UNESP, WDCM 1182), *L. casei* SJRP38, *L. casei* SJRP66, *L. casei* SJRP146, *L. casei* SJRP169. These four *Lactobacillus* strains were previously isolated from the processing of water-buffalo mozzarella cheese, identified by 16S rRNA gene sequencing, and characterized (Silva et al. 2015; Salotti de Souza et al., 2019).

All *Lactobacillus* strains were cultured in MRS broth (Difco™, Leeuwarden, Netherlands) and stored in 20% sterile glycerol (*v/v*) at -80 °C. Each strain was thawed and reactivated in MRS broth (Difco™, Leeuwarden, Netherlands) and incubated aerobically for 24 h at 37 °C.

2.2. In vitro growth kinetics evaluation under fermented sausage process conditions

The capability of *L. casei* strains to grow under fermented sausage process conditions, such as different pH values (4.5; 5.0 and 5.5), 150 ppm sodium nitrite, 150 ppm sodium nitrate, 2% probiotic fiber – FOS and sodium chloride (2.0%; 3.75% and 5%) in MRS broth was performed *in vitro,* like used by Nascimento et al. (2019). After 24 h of incubation in MRS broth, as mentioned in 2.1 (Difco™, Leeuwarden, Netherlands), the optical density (OD) recorded at λ = 600 nm (OD_{600nm}) was adjusted to 0.1 at 37ºC. This test was conducted in a sterile flat-bottom 96-well microtiter plates. In total 20 µL of each culture of *L. casei* were inoculated into 180 µL of MRS modified broth (Difco™, Leeuwarden, Netherlands) in 96-well flat bottom microtiter plates and incubated anaerobically at 15°C and 25°C. OD_{600nm} was recorded every 2 h during 10 h and after 24 h, using a microtiter plate reader (Ez Read 2000, BioChrom, Cambridge, UK). This experiment was performed in different trials in triplicate (n=9).

2.3. Low fat Italian type Salami manufacturing

The strains which resulted in higher growth under fermented sausage process conditions were selected for the production of low-fat Italian type salami. The *L. casei* strains were grown in MRS broth at 37°C/24h. Then, 2 mL aliquot of the culture were centrifuged in 8.000 *g* for 10 min at 4° C, washed and resuspended in saline solution (0.85%). This inoculum was stored refrigerated (2° C) until their use in the low-fat Italian type salami production. The initial count of each inoculum was standardized to 10 log CFU/mL.

Four formulations were produced in two different manufacturing times (January and February 2019): **C** (control) - 80 g/100g of fresh pork shoulder (moisture 74 g/100g, fat 6 g/100g) and 20 g/100g of pork back fat (moisture 11 g/100g, fat 82 g/100g); **FOS** (Fat reduced of 25g/100g with 2 g/100g of FOS), **FOS_66** (Fat reduced of 25 g/100g with 2 g/100g of FOS and *L. casei* SJRP66) and **FOS_169** (Fat reduce of 25 g/100g with 2 g/100g of FOS and *L. casei* SJRP169)- 80 g/100g of fresh pork shoulder, 15 g/100g of pork back fat and 5 g/100g of FOS mixture (2 g/100g of FOS + 3 g/100g of water) + *L. casei*. In all formulations, others ingredients were added: sodium chloride (2.5 g/100g), sucrose (2 g/100g), sodium nitrite (0.015 g/100g), sodium nitrate (0.015 g/100g), garlic (0.2 g/100g), white pepper (0.2 g/100g), nutmeg (0.3 g/100g), sodium erythorbate (0.05 g/100g), and a starter culture (0.025 g/100g- Bactoferm T-SPX (Chr. Hansen, Hoersholm, Denmark)) composed of *Pediococcus pentosaceus* and *Staphylococcus xylosus*. All the ingredients were mixed using an automatic blender for approximately 5 min. The probiotic inoculum was added in at the end of the process. After, the mixture was stuffed in cellulose cases (diameter of 50 mm) and separated into pieces of 15 cm (approximately 150 g) for each treatment. The temperature and relative humidity parameters (TºC and RH %) were controlled following the procedure: 25°C/90% for three days; 23°C/88% for three days; 20°C/85% for three days; 18°C/80% for five days and 15°C/75% until day 18.

2.4. Ripening evaluation

The pH value was measured with a penetration probe in a PG 1800 digital pH meter (Gehaka, São Paulo, Brazil). Five sausages (with an initial weight of approximately 250 g) from each treatment were initially weighed. The same samples were reweighed on days 3, 6, 12 and 18. Weight losses were expressed as the percentage of the initial weight (Glisic et al., 2019). Moisture percentage was calculated by a weight loss experiment keeping the sample in a laboratory oven at 105 ºC until constant weight. All analyses were performed in triplicate.

2.5. LAB total and probiotic L. casei count

The samples were weighed (10 g) in sterile plastic containers and mixed with 90 mL 0.85% saline water for 2 min. The total of LAB was counted using MRS agar (Difco™) incubated at 37°C/48 h under anaerobic conditions. *Pediococcus pentosaceus (*data not show) was counted using a modified MRS agar (Difco™), added with tetracycline (Inlab, Alamar Tecno-Científico Ltda.) to ensure inhibition of growth of *Lactobacillus* sp. as described by Macedo, Pflanzer, Terra, & Freitas (2008). The count of probiotic *L. casei* added in the Italian type salami was determined by the difference from the total of LAB count and the *Pediococcus pentosaceus* count, as described by Macedo et al. (2008). All analyses were performed in triplicate during ripening time on days 0, 3, 6, 12 and 18 after the stuffing process.

2.6. Technological characterization

The degree of lipid oxidation was evaluated by measuring the TBARS value, using a standard curve of malonaldehyde (MA) and expressed as mg MA/kg of sample, according to Vyncke (1970) with modifications.

The color evaluation was performed using a ColorFlex45/0 spectrophotometer (Hunterlab, Reston, USA), observation angle of 10 º, illuminant D65 and Universal software version 4.10. The CIELAB color specification system was used and the color coordinates determined were lightness (L*), redness (a*) and yellowness (b*).

The texture parameters, hardness (N), cohesiveness, springiness (mm) and chewiness (N mm^{-1}) were performed at room temperature (25 ºC) as described by Bourne (1978), using TA-XT/Plus/50 texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a cylindrical probe 36 mm in diameter. The samples of fermented sausage were cut into slices of

2 cm and were axially compressed in two consecutive cycles of 50% of compression, at a constant test speed of 1 mm/s (n=10).

2.7. Statistical analysis

The results of viability under fermented sausage process conditions were analyzed through the average calculation and standard error of mean. The results are represented as an average of 9 readings.

Four formulations (C, FOS, FOS_66 and FOS_169**)** during five ripening times (0, 3, 6, 12 and 18 days) in two different manufacturing times were analyzed for different dependent variables.

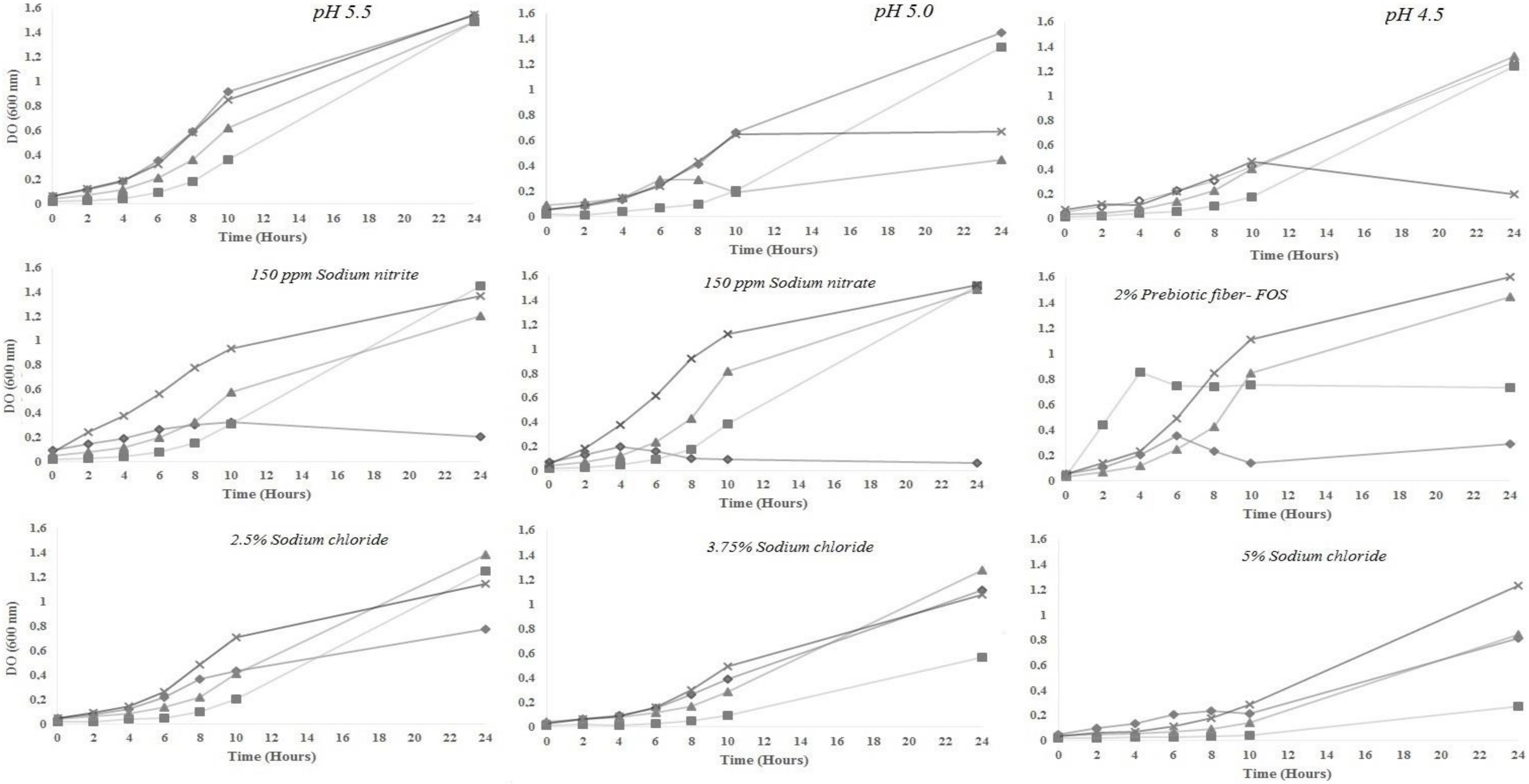
The data from the application of *L. casei* SJRP in low fat Italian type salami during the ripening time were analysed using the one-way ANOVA randomized complete block design using the generalized linear model (GLM). All the results were expressed as the mean values and the standard error of the mean. Mean comparisons were assessed by the Tukey test at a significance level of 5% at the same ripening time.

3. Results and Discussion

3.1. In vitro growth kinetics evaluation in LAB

At 25 °C and pH 5.5, all the probiotics strains showed a similar growth rate after 24 h. On pH value 5.0, the *L. casei* strains SJRP169 and SJRP146 showed a poor growth compared to the others (Figure 1), while the pH 4.5 was a limiting factor for the growth of *L.* SJRP169. On the other hand, at 15 ºC (Figure 2), the pH variation does not appear to have a significant effect on growth rate. Since the low temperature decreased the growth of all LAB tested, confirming the low temperature as a limiting factor (Ammor and Mayo, 2007). Among the strains, *L. casei* SJRP66 and *L. casei* SJRP169 showed higher viability at pH 4.5 and low temperature (15ºC), which makes it more robust for use in Italian type salami, since these conditions are usual during salami production. These strains also tolerated harsh conditions, such as the simulated GI tract conditions under pH of 2.5 with viability > 6 log CFU/mL (Casarotti et al., 2017), which is one of the criteria for LAB be consider potentially probiotic.

At the room temperature (Figure 1), the addition of 150 ppm of sodium nitrite or sodium nitrate proved to be a growth limiting factor, especially for *L. casei* SJRP38. The other strains presented a higher growth rate in the presence of these chemical additives. In contrast, at 15 ºC (Figure 2) none of the tested *L. casei* strains showed DO_{600nm} > 0.4. The capacity to tolerate the sodium nitrite concentrations has already been reported as an important factor for probiotics viability in fermented meat products (Rouhi et al., 2013). However, with the trend towards healthier meat products, more and more researchers are testing the production of fermented meat sausages with probiotic LAB with reduced nitrite or nitrite free content (Ben Slima et al., 2017; Coelho et al., 2019).



Caption:

Lactobacillus casei SJRP 38 — *Lactobacillus casei* SJRP 66 — *Lactobacillus casei* SJRP 146 — *Lactobacillus casei SJRP 169*

Figure 1 - Comparison of growth of *L. casei* SJRP38, *L. casei* SJRP66, *L. casei* SJRP146 and *L. casei* SJRP169 in MRS modified for 24 h at 25ºC.

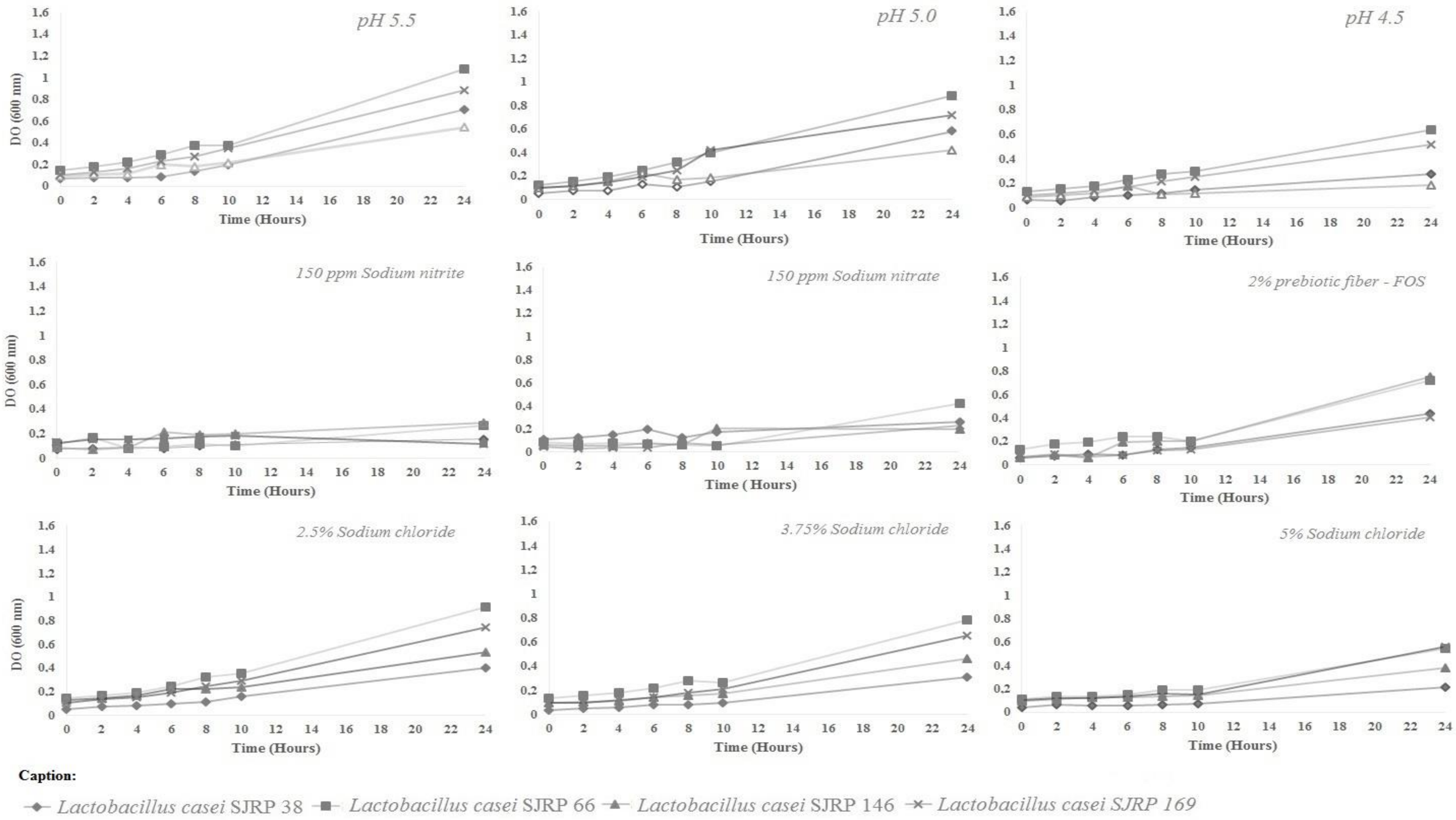


Figure 2 - Comparison of growth of *L. casei* SJRP38, *L. casei* SJRP66, *L. casei* SJRP146 and *L. casei* SJRP169 in MRS modified for 24 h at 15ºC.

The addition of 2% FOS in the MRS broth showed a positive effect on the *L. casei* viability depending on the incubation temperature of *L. casei* SJRP146 and SJRP169 presented a higher growth rate at 25ºC, and *L. casei* SJRP66 showed a median growth rate, while at 15ºC, *L. casei* SJRP66 and SJRP146 had the highest viability. FOS is a prebiotic fiber that should increase the LAB viability in stress situation, favoring the growth of beneficial bacteria (Farias, Araújo, & Neri-Numa, 2019). The interaction of FOS and different probiotic LAB strains have been tested by several scientific groups (Bambace, Victoria, & Moreira, 2019; Bersaneti, Garcia, Mali, & Celligio., 2019; Duysburgh et al., 2019; Bis-Souza et al., 2020).

The combine of FOS and probiotic *Lactobacillus* spp. can improve several physiological effects to the host including the absorption of minerals and the intestinal function. Also, it can regulate the glucose and lipid metabolism and reduce the risk of development of colon cancer (Farias et al., 2019). All these effects are a consequence of the stimulus to the growth of the beneficial microbiota that the prebiotic fiber exerts in the pass through the GI tract. These compounds are fermented by the gut microbiota and produce short chain fatty acids (Salazar, García, & Selgas, 2009). Therefore, the prebiotic is recognized by the growth of the probiotic population in the human gut (Muñoz et al., 2012).

L. casei SJRP146 showed the higher growth potential in the presence of 2.5 g/100g and 3.75 g/100g NaCl, at 25 ºC. However, the strain with the most halophilic characteristic (at 5% NaCl) was *L. casei* SJRP169, while *L. casei* SJRP66 showed the lowest tolerance to high concentration of NaCl. Be halotolerant is an important characteristic for the probiotic strains used on the production of fermented meat products (Penna et al., 2014; Amaral et al., 2016).

The fermentation process in a meat product is responsible to the formation of several compounds accountable for the sensory and technological characteristics of the final product, including the microbiological safety (Lorenzo, Montez, Purriños, & Franco, 2012; Leite et al., 2015; Lorenzo, Gómez, Purriños, & Fonseca, 2015; Bis-Souza et al., 2020).

According to Rouhi et al. (2013), the stress caused to the probiotic strain when submit to different temperatures and high sodium concentration could affected the LAB viability. Similar result was observed at 15 °C (Figure 2) whereas all *L. casei* strains showed a lower growth rate compared to the same NaCl concentration at room temperature (Figure 1). Since these parameters are considered limiting to the growth of microorganisms, it is crucial to perform *in vitro* tests and ensure the choice of the most appropriate probiotic strain for this type of product submitting the strains by different temperatures, salt concentration and pH (Ammor and Mayo, 2007).

Due to the complexity of the meat matrix and the probiotic sensitivity to the severe conditions of the fermented sausage process, the *in vitro* test is not enough to guarantee a correct LAB selection (Bis-Souza et al., 2019a). Considering their positive outcome, *L. casei* SJRP66 and SJRP169 were chosen for application in low-fat Italian type salami. This evaluation will enhance the knowledge about their effect on the technological and microbiological properties of the product.

3.2. Ripening evaluation of Italian type salami

The addition of the *L. casei* SJRP66 and *L. casei* SJRP169 showed no effect (P > 0.05) in the pH decrease during the ripening time compared to control (C) sample (Table 1). The low pH value in a fermented sausage can inhibit the growth of spoilage and pathogenic organisms, which ensures greater microbiological quality and safety (Lorenzo, Gómez, & Fonseca, 2015). Therefore, the selection of an acid-producing LAB strains plays a significant role in fermented meat products (Zhang et al., 2017). Distinct effect was observed in the addition of *L. casei* LOCK 0900 in dry fermented sausage, since the probiotics samples presented a lower pH value compared to control treatment (Wójciak, Dolatowski, Kolozyn-Krajewska, & Trzaskowska 2012). Other authors reported similar pH decrease during ripening time of fermented sausage, reaching pH values near to 4.6 (Dominguez, Munekata, Agregan, & Lorenzo, 2016; Ba et al., 2018; Montanari et al., 2018).

The fat substitution increased (*P*<0.05) the pH value compared to the other samples. FOS sample showed the highest pH value (*P*<0.05) during all the ripening time; similar effect was reported by many authors (Vasilev et al., 2017; Glisic et al., 2019; Bis-Souza et al., 2020).

The moisture content (Table 1) of all formulations decreased during the ripening time, due to the dehydration effect that occurs in dry fermented meat products (Fonseca, Gómez, Domínguez, & Lorenzo, 2015). C sample presented highest values of moisture (*P*<0.05) compared to the samples from other samples. Fat reduction and addition of FOS facilitates water loss, and consequently, increased the weight loss (Table 1). The total weight loss (near to 39%) at the end of ripening time was similar to values reported for fermented sausage (Backes et al., 2013; Van Ba et al., 2016; Savoldi et al., 2019).

These results may be associated with the ease of water loss in fermented sausages formulations with lower fat content and added with short chain soluble fibers. As reported by Salazar et al. (2009) the short chain FOS appears to increase the loss of water during ripening time. The significant difference between the pH values at the end of the ripening time may also explain the higher weight loss of the FOS sample, since its pH value (4.91) is near to the protein isoelectric point.

Table 1 - Effect of fat replacement in the ripening process of salami type Italian added with probiotic strains.

	Treatments	Ripening Time (Days)					SEM
		0	3	6	12	19	
pH	**C**	6.01	4.76^{b}	4.72^{b}	4.77^{b}	4.79^{b}	0.09
	FOS	5.99	4.87^{a}	4.84^{a}	4.84^{a}	4.91^{a}	0.09
	FOS_66	5.96	4.75^{b}	4.66^{b}	4.74^{b}	4.76^{b}	0.09
	FOS_169	5.95	4.81^{b}	4.65^{b}	4.71^{b}	4.79^{b}	0.09
SEM		0.01	0.01	0.01	0.01	0.02	
P value		0.239^{ns}	0.000	0.000	0.000	0.001	
Moisture (%)	**C**	56.36^{b}	55.71	49.99	48.07^{a}	44.11^{a}	1.13
	FOS	60.42^{a}	56.35	52.50	43.46^{b}	38.22^{b}	1.91
	FOS_66	60.56^{a}	58.52	50.89	43.68^{b}	37.12^{b}	2.08
	FOS_169	60.85^{a}	57.90	52.64	43.83^{b}	38.19^{b}	1.97
SEM		0.63	0.52	0.64	0.62	0.86	
P value		0.013	0.187^{ns}	0.422^{ns}	0.006	0.003	
Weight loss (%)	**C**	-	9.45	15.97	25.16^{b}	30.93^{b}	2.03
	FOS	-	10.24	18.33	31.06^{a}	36.61^{a}	1.62
	FOS_66	-	9.04	17.67	31.02^{a}	38.55^{a}	1.94
	FOS_169	-	8.59	17.05	30.70^{a}	37.31^{a}	1.98
SEM		-	0.37	0.51	0.50	0.61	
P value		-	0.474^{ns}	0.423^{ns}	0.000	0.000	

^{a,b} Mean values in the same column (different formulation in the same ripening time) with different letter presented significant differences in the Tukey Test; SEM: standard error of mean; ^{ns}: not significant $P > 0.05$. C: control treatments with 20% of pork back fat; FOS: fat reduced of 25% with 2% of FOS; FOS_66: fat reduced of 25% with 2% of FOS and *L. casei* SJRP66; FOS_169: fat reduce of 25% with 2% of FOS and *L. casei* SJRP169.

3.3. LAB count

Regardless of the BAL count (Figure 3. A), at the beginning of the fermentation process (day 0 and day 3), all the treatments samples showed similar values (6 log CFU/g and 7 log CFU/g, respectively). At the end of the ripening process, C and FOS samples showed similar viable cell count, above 7 Log CFU/g.

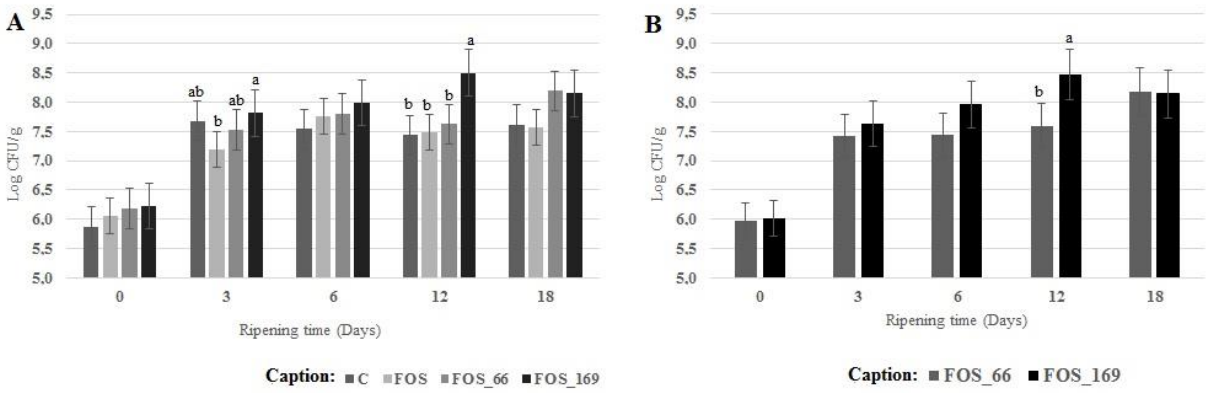


Figure 3 - BAL count (A) and *L. casei* count (B) during ripening time of low-fat Italian type salami. C: control treatments with 20% of pork back fat; FOS: fat reduced of 25% with 2% of FOS; FOS_66: fat reduced of 25% with 2% of FOS and *L. casei* SJRP66; FOS_169: fat reduce of 25% with 2% of FOS and *L. casei* SJRP169. . ^{a,b} Different letter in the same ripening time presented significant differences in Tukey test ($P<0.05$). Mean and standard error.

*L. cas*ei SJRP66 and *L. casei* SJRP169 presented high viability in the low-fat Italian type salami, reaching 8 Log CFU/g after 19 days of ripening (Figure 3. B). Similar counts of viable cells were reported by Ruiz, Villanueva, Favaro-Trindade, & Contreras-Castillo (2014) when used a *L. acidophilus* and *B. lactis* as probiotic strains in Italian salami, by Rubio, Martín, Aymerich, & Garriga (2014) using the *Lactobacillus rhamnosus* CTC1679 in low-acid fermented sausages (*fuets*), and by Coelho et al. (2019), who developed a dry-fermented sausage added of *L. paracasei* LPC02 and prebiotic lactulose.

The prebiotics can improve human health by controlling the imbalance of the gut microbiota by the stimulation of *Lactobacillus* spp. growth. According to Ahmadi et al. (2019)

and Faria et al. (2019) a diet containing prebiotic compounds improves glucose intolerance, insulin resistance, improved immunity and modulation of metabolic, cardiovascular and inflammatory biomarkers, increases the levels of short chain fatty acids in rat intestine and reduces the intestinal hyperpermeability and inflammatory markers of the mucosa.

In order to result in beneficial effect on the host, it is necessary to ensure the presence of high counts of probiotic cells, ensuring its viability to compete with the endogenous microbiota until the end of the fermented sausage processing (Ruiz-Moyano et al., 2011; Cavalheiro et al., 2015).

Short chain FOS is a compound that is neither digested nor absorbed in the human gut (Muñoz et al., 2012). So, the probiotic microbiota, as *Lactobacillus* spp. can fermented this fiber and produce short chain fatty acids that are related to human functional health. Thus, the consume of a fermented meat sausage that can provide this symbiotic benefit into consumer health is a breakthrough in the food technology.

3.4. Technological characterization

The addition of potential probiotic strains in the low-fat Italian type salami showed no effect on the lipid oxidation (TBARS value) regardless the fat content (Table 2). Similar results were reported by Libera et al. (2015) when used *Bifidobacterium animalis* ssp. *lactis* BB-12 in dry-cured pork neck. Similar results were found by Yalınkılıç, Kaban, & Kaya (2012) in sausage added with citrus fiber. It is worth noting that the amount of malonaldehyde formed during the ripening process of the Italian type salami added with probiotic strains resulted in TBARS values much lower than 2.0 mg MDA/kg, which is accepted as the deterioration level and sensory detection (Greene & Cumuze, 1982; Carvalho et al., 2019).

The fat level had a significant correlation with the L^* value in low-fat Italian type salami (Table 2), which agree with reported by several authors (Salazar et al., 2009; Jung et al., 2018;

Bis-Souza et al., 2019b). C samples showed the highest value (*P*<0.05) for *L** comparing to the samples from other treatments. The addition of the probiotic strains showed effect (*P*<0.05) only comparing FOS_169 with FOS samples. So, the use of FOS and *L. casei* SJRP169 appears to result in a darker Italian type salami than the formulation only added of FOS.

Table 2 - Effect on TBARS, color and texture parameters in low-fat Italian type salami with probiotic strains.

	C	FOS	FOS_66	FOS_169	SEM	*P value*
TBARS (mg MDA/kg)	0.288	0.235	0.239	0.233	0.008	0.059^{ns}
L*	59.90^{a}	47.51^{b}	46.39^{bc}	43.85^{c}	0.730	0.000
a*	12.62^{b}	14.07^{a}	13.66^{ab}	13.54^{ab}	0.186	0.041
b*	12.91^{a}	10.80^{b}	10.52^{b}	9.84^{b}	0.196	0.000
Hardness (N)	66.29^{c}	147.60^{b}	213.97^{a}	201.67^{a}	6.75	0.000
Cohesiveness	0.515^{b}	0.594^{a}	0.596^{a}	0.613^{a}	0.008	0.000
Springiness (mm)	0.302^{b}	0.416^{a}	0.445^{a}	0.434^{a}	0.008	0.000
Chewiness (N/mm)	11.82^{c}	36.18^{b}	56.73^{a}	53.82^{a}	2.109	0.000

^{a,b,c,} Mean values in the same line with different letter presented significant differences in the Tukey Test; SEM: standard error of mean; ^{ns}: not significant *P* > 0.05. C: control treatments with 20% of pork back fat; FOS: fat reduced of 25% with 2% of FOS; FOS_66: fat reduced of 25% with 2% of FOS and *L. casei* SJRP66; FOS_169: fat reduce of 25% with 2% of FOS and *L. casei* SJRP169.

In contrast, the intensity of the red color (*a** value) was not affected (*P*>0.05) by the addition of the *L. casei* strains, only the reduction of fat level affected significantly this color coordinate. FOS samples showed higher values (*P*<0.05) than C. Similar findings were reported by other authors (Alejandre, Poyato, Ansorena & Astiasaran, 2016; Ba et al., 2018; Glisic et al., 2019).

The reduction in fat content decrease (P<0.05) the intensity of the yellow color (b* value). Similar results were reported by Keenan, Resconi, Kerry, & Hamill (2014), Berizi et al. (2017) and Bis-Souza et al. (2019b) when using prebiotic fiber as a fat substitute. The results of lightness and yellowness could be associated with the drying process (reducing of the moisture) that induced the retraction of the fiber which led to darker product (Glisic et al., 2019).

The 25 g/100g of fat reduction in the low-fat Italian type salami increased (P<0.05) hardness and chewiness compared to the C treatment. The addition of probiotic strains also increased hardness (P>0.05) in low-fat Italian type salami. Similar results were reported for other fermented products (Lorenzo, & Franco, 2012; Domínguez et al., 2016; Menegas, Pimental, Garcia, & Prudencia, 2017), since fat reduction is usually associated with hardness and chewiness increase in fermented sausage. Treatments containing probiotic strains showed the highest hardness value (P>0.05) probably due to more pronounced moisture and weight losses, which occurred during dry process, as consequence of the pH value near to the isoelectric point of the meat matrix.

Regarding cohesiveness and springiness, the partial substitution of fat and the use of *L. casei* strains in the fermented process increased (P>0.05) those texture parameters. This behavior was also reported when gel inulin-based were used as fat replacer in dry fermented sausage (Glisic et al., 2019) and in low-fat Spanish Salchichón added with FOS and probiotic strains (Bis-Souza et al., 2020).

The addition of probiotic strains in fermented meat products can present an impact on the technological properties of the final product. So, it is very important to evaluate those alterations, especially in texture, that could result in the rejection by the consumers (Cavalheiro et al., 2015). Thus, the texture profile of the Italian type salami was evaluated after 19 day of ripening time, in the final product (Table 2).

Additional evaluations are important to better understand the effect of FOS and probiotic *L. casei* in low-fat Italian salami, such as sensory evaluation, proteolysis, production of volatile compounds and the fatty acid profile.

4. Conclusion

The conditions applied (temperature, pH and salt variations, concentration of nitrite, nitrate and FOS) were limiting for *L. casei* strains growth. The most resistant probiotic strains (*L. casei* SJRP66 and *L. casei* SJRP169) when added with prebiotic fiber (FOS) presented good technological features during the production of salami, despite some differences in texture parameters. Then, the low-fat Italian type salami produced with addition of FOS and probiotic *L. casei* strains can present healthier appeal.

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CAPÍTULO 4

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Impact of fructooligosaccharides and probiotic strains on the quality parameters of low-fat Spanish *Salchichón*.

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ABSTRACT

The substitution of dietary fiber and probiotic strains to reduce fat content of fermented sausages has been used for the development of innovative and healthier meat products. For this study, pork back fat was partially replaced by fructooligosaccharides (FOS) and the probiotic strains *Lactobacillus paracasei* and *Lactobacillus rhamanosus*. The fat replacement resulted in a significant decrease ($P \leq 0.05$) in fat content (29%) compared with the control formulation (no fat substitution). The addition of FOS did not have a significant effect on microbial counts; however, reductions in *Enterobacteriaceae* and yeast were observed when *Lactobacillus* strains were also incorporated. The inclusion of FOS and probiotic strains did not show any significant effect on lipid oxidation and proteolysis. The partial fat replacement and the addition of *Lactobacillus rhamanosus* GG as probiotic strain in Spanish *Salchichón* can be considered a successful reformulation strategy for the meat product market.

Keywords: fat substitutes, fermented meat product, healthy, functional, prebiotic fiber.

1. INTRODUCTION

In recent years, several strategies have been tested to develop healthier meat products without affecting sensory, technological and textural properties. The partial substitution of pork fat by functional ingredients, such as prebiotic dietary fiber, has been one of the highlights in meat product research. The use of probiotic strains in consortium with the traditional starter culture in fermented meat sausages has been recognized as a technologically feasible and effective strategy for the development of innovative products (Bis-Souza, Barba, Lorenzo, Penna, & Barretto, 2019). Fructooligosaccharides (FOS) are short chain carbohydrates that currently dominate the prebiotic category and are recognized for their functional food effects. Carbohydrates with prebiotic properties present a high resistance to digestion and absorption by the gastrointestinal tract resulting in a reduced caloric content (Ruiz-Aceituno, Hernandez-Hernandez, Kolida, Moreno, & Methven, 2018). When a prebiotic is administered in combination with probiotic strains, after their ingestion they can act synergically in the human intestines (Casarotti, Borgonovi, Batista, & Penna, 2018) and ensure the viability of these beneficial microorganisms. This can bring health benefits to the gastrointestinal tract of the host (Gibson *et al.,* 2017).

Some other studies have reported the positive effect of prebiotic carbohydrates, such as FOS, in low-fat meat products to improve the technological parameters and sensory characteristics (Felisberto, Galvão, Picone, Cunha, & Pollonio, 2015). In addition, research has been carried out to develop functional meat products using probiotic strains in fermented sausage process (Ba *et al.,* 2018; Rubio, Jofré, Aymerich, T., Guàrdia, & Garriga, 2014b; Rubio, Martín, Aymerich, & Garriga, 2014a; Ruiz- Moyano *et al.,* 2011; Slima *et al.,* 2018).

Traditional Spanish *Salchichón* is a dry-ripened sausage made exclusively with lean pork, pork back fat, salt and spices (Fonseca, Gómez, Domínguez, & Lorenzo, 2015). This type of dry-fermented sausage is traditionally manufactured without adding a starter culture. However, the use of a starter culture could guarantee a process, which standardizes sensorial and technological characteristics in a shorter ripening time (Benito *et al.,* 2007). In the fermented process, strains of lactic acid bacteria (LAB) are responsible for the acidification, resulting from the production of organic acids, improving flavor, taste and biological safety (Ba *et al.,* 2018). Most commercial fermented sausages present a combination of starter cultures to guarantee their unique flavors and *Lactobacillus, Staphylococci* and/or *Micrococci* are generally used (Cheng, Liu, & Zhang, 2018). Most of the bacteria that have been considered for use as probiotics belong to the genus *Lactobacillus* and *Bifidobacterium* (Bis-Souza et al., 2019). Probiotic strains of *Lactobacillus paracasei LPC02* and *Lactobacillus plantarum* NJAU-01 have already been applied in the processing of fermented sausage meat with improvements in technological characteristics (Coelho et al., 2019; Ge et al., 2019). The sausage matrix is considered adequate for carrying probiotic bacteria (Ammor & Mayo, 2007).

The present study aimed to evaluate the influence of the addition of fructooligosaccharides and two different probiotic commercial strains on the technological, microbiological and sensory parameters of low-fat Spanish *Salchichón*.

2. MATERIAL AND METHODS

2.1. Prebiotic and probiotic materials

The prebiotic fiber used as fat substitute was NutraFlora® P95 soluble prebiotic fiber (short-chain fructooligosaccharides (FOS) - moisture 4%; total dietary fiber 95%) (Ingredion, Westchester, USA). The commercial starter culture Bactoferm T-SPX® (*Pediococcus pentosaceus* + *Staphylococcus xylosus),* (Chr-Hansen, Hørsholm, Denmark) and the commercial probiotic strains Lyofast BGP 1® (*Lactobacillus paracasei*) (SaccoSystem, Cadorago, Italy) and LGG® (*Lactobacillus rhmanosus* GG) (Chr-Hansen, Hørsholm, Denmark) were used.

2.2. Preparation of low-fat Spanish Salchichón

Four different formulations were manufactured in the pilot plant of the Meat Technology Center of Galicia (San Cibrao das Viñas, Ourense, Spain): A control formulation (denominated CON) without probiotic strains or FOS; Low-fat with FOS 2 g/100 g added (denominated FOS); Low-fat with FOS 2 g/100 g and *Lactobacillus paracasei* added (FOS.BGP1); Low-fat with FOS 2 g/100 g and *Lactobacillus rhmanosus* GG added (FOS.GG). The control (CON**)** was prepared using lean pork (80 g/100 g) and pork back fat (15 g/100 g) from Celta pigs, "542 Salchichon" supplement (5 g/100 g) (Laboratorios Ceylamix, Valencia, Spain) composed, in unknown proportions, of sugar, salt, dextrin, spices (black and white pepper and nutmeg), milk protein, monosodium glutamate (E621), phosphates (E450i and E451i), sodium erythorbate (E316), potassium nitrate (E252) and coloring (E120), and the commercial starter culture Bactoferm T-SPX (0.025 g/100g). The low-fat formulations were produced using lean pork (80 g/100 g), pork back fat (10 g/100 g), cold water to homogenize (3 g/100 g), FOS (2 g/100 g), "542 Salchichon" supplement (5 g/100 g), the commercial starter culture Bactoferm T-SPX (0.025 g/100 g) and the probiotic strains (0.01 g/kg), according to each formulation.

The sausage manufacture was carried out according to the method described by (Fonseca *et al*., 2015) with some modifications. Six replicate samples from each formulation and batch were taken at day 0, 3 and after 15, 30 and 45 days of ripening. The four formulations were manufactured with the same ingredients, formulation and technology in two batches: one in March and one in April of 2018. All the physicochemical, technological and microbiological evaluation were performed in sextuplicate.

2.3. Physicochemical characteristics and proximate composition

Physicochemical parameters (pH, colour, and texture profile analysis (TPA) and proximate composition (moisture, total fat, protein and ash) were measured following the method described by Lorenzo, Munekata, Pateiro, Campagnol, & Domínguez (2016a). For that, each Spanish *Salchichón* was cut into three pieces of 2 cm which were used for each analysis. Water activity (a_w) was measured using a Decagon Aqualab water activity meter (Decagon Devices Inc., Pullman, USA), previously calibrated with sodium chloride.

2.4. Lipid oxidation

Lipid stability was evaluated using the TBARS index following the method described by Pateiro, Bermúdez, Lorenzo, & Franco (2015). The values were expressed as mg MDA/kg sample.

2.5. Free amino acid analysis

Free amino acids were extracted following the procedure described by Pérez-Palacios, Ruiz, Barat, Aristoy, & Antequera (2010) with the modifications proposed by Lorenzo *et al.* (2015). Results of free amino acid content were expressed as mg/100 g of dry matter.

2.6. Microbiological analysis

For the microbiological analysis, 10 g of Spanish *Salchichón* were aseptically weighed in a sterile plastic bag, homogenized with 90 mL of 0.1% (w/v) sterile peptone water (Oxoid, Basingstoke, UK), containing 0.85% NaCl and 1% Tween 80 as emulsifier, for 2 min at 20-25

°C in a Masticator blender (IUL Instruments, Barcelona, Spain). For each sample, appropriate serial decimal dilutions were prepared in sterile peptone water (0.1%). As microbiological quality indicators, the counts of lactic acid bacteria (LAB), yeasts, *Enterobacteriaceae* and mesophilic aerobes microorganisms were tested.

The counts of Mesophilic aerobic bacteria and *Enterobacteriaceae* were carried out using the TEMPO system (BioMerieux, Marcy l'Etoile, France) based on the Most Probable Number technology. The TEMPO system consists of 2 ergonomic stations, a preparation station for the filling of the disposable cards (each card contains 16 x 3 tubes) with culture medium inoculated with the samples and a reading station where the reading is done after the incubation time stipulated for each test. Aerobes were incubated at 30 °C and *Enterobacteriaceae* at 35 °C for 24 hours.

LAB were counted after inoculation on Man Rogosa Sharpe agar (Oxoid, Basingstoke, UK) (pH 5.6), and incubation at 30 °C for 5 days. For the yeast count, 0.1 mL of each mother dilution and the successive decimal dilutions were inoculated on the surface of Rose Bengal Chloramphenicol Agar plates (Oxoid, Basingstoke, UK), Chloramphenicol Selective Supplement added (Oxoid, Basingstoke, UK) and incubated at 25 °C for 5 days. The microbiological data were expressed as logarithms of the number of colony forming units (Log CFU/g).

2.7. Sensory analysis

The Ranking Sensory Test (UNE-ISO 8587-2010) was performed at the Meat Technology Center of Galicia (San Cibrao das Viñas, Ourense, Spain) with 30 trained panelists. The testing was carried out in a room equipped with individual tasting booths according to ISO 8589:2007 under white light. Water and bread without salt were available for rinsing the mouth between samples. Slices (5 mm) were obtained using a commercial slicing machine and were served to the panelist at room temperature. The four samples were presented to participants following a balanced order for each of them (Macfie, Bratchell, Greenhoff, & Vallis, 1989). For the purpose of expressing their preferences, the panelists were asked to classify the samples from the most preferred (score 1) to the least preferred (score 4). The different formulations were evaluated for appearance, texture, flavour and overall acceptance. The samples were served in randomized order and individually three-digit coded for all panelists. To check if the differences between the samples are significant, a total score for each sample was obtained and the Friedman test was applied to the data.

2.8. Statistical analysis

A total of 240 sausages [six low-fat fermented sausage samples × four formulations (CON, FOS, FOS.BGP1, FOS.GG) × five ripening times (0, 3, 15, 30, 45 days) × two different processing batches (March and April 2018) were analyzed for different dependent variables.

For the statistical treatment, an analysis of variance (ANOVA) with a mixed-model was performed for all variables considered in the study. These parameters were set as dependent variables, while ripening time and formulation were included in the model as fixed effects, while the different batches and replicates were considered as random effects. Duncan's test was used to assess the pairwise differences between least square means wherein such differences were considered significant if $P \leq 0.05$. The values were expressed as mean values and standard error of the mean (SEM). The significant differences between samples in the ranking test were studied using the Friedman test, and paired significances were characterized using the Minimum Significant Difference as criterion ($\alpha = 0.05$). All statistical analysis was performed using Statistic 7.0 software.

3. RESULTS AND DISCUSSION

3.1. Physicochemical parameters

As expected, significant differences ($P \leq 0.05$) were observed for fat content (Table 1). A decrease of 29.3%, 31% and 32.7% in the fat content value was observed when compared FOS, FOS.BGP1 and FOS.GG batches with CON group, respectively. Regarding protein and ash contents, no significant differences were observed between the formulations. In general, these results are similar to those reported by other authors for dry-cured sausages (Dominguez *et al.*, 2016).

Table 1. Effect of the partial replacement of pork back fat by FOS and the addition of probiotic strains on proximate composition of low-fat fermented sausages (g/100g).

	Batches				SEM	*P value*
	CON	FOS	FOS.BGP1	FOS.GG		
Protein	16.87	16.68	16.51	16.55	0.06	0.144[ns]
Fat	19.70[a]	13.92[b]	13.58[b]	13.26[b]	0.42	0.001
Ash	2.81	2.99	2.82	2.82	0.04	0.293[ns]

Batches: CON: Control without probiotic strains and without FOS; FOS: Low-fat added with FOS 2 $g.100g^{-1}$; FOS.BGP1: Low-fat added with FOS 2 $g.100g^{-1}$ and *Lactobacillus paracasei*; FOS.GG: Low-fat added with FOS 2 $g.100g^{-1}$ and *Lactobacillus rhmanosus* GG. [a,b,c] Mean values in the same row with different case letter presented significant differences. SEM: standard error of mean

The low-fat Spanish *Salchichón* showed significant ($P\leq0.05$) differences in pH values from 3 days of ripening until the end of process. The trend in fermented sausages is for pH to decrease over time, regardless of the formulation (Table 2). This decrease in pH values occurs due to the accumulation of organic acids resulting in carbohydrate breakdown during fermentation (Cheng *et al.,* 2018; Pateiro *et al.,* 2015). Samples that contained probiotic strains in their formulation (FOS.BGP1 and FOS.GG) presented significantly ($P\leq0.05$) lower pH values than FOS samples after 45 days (Table 2), probably because they intensified the fermentation process, decreasing the pH values (Cavalheiro *et al.,* 2015). Similar results were found by other authors in dry-fermented sausages manufactured with *Lactobacillus rhamnosus* and *Lactobacillus paracasei* (Erkkilä, Suihko, Eerola, Petäjä, & Mattila-Sandholm, 2001; Salazar *et al.,* 2009). However, this decrease in pH values could lead to unpleasant physicochemical changes, especially when the values are less than 5.0 (Työppönen, Petäjä, & Mattila-Sandholm, 2003).

Table 2. Effect of the partial replacement of pork back fat by FOS and the addition of probiotic strains on pH, water activity (a_w) and moisture content (%) of low-fat fermented sausages during ripening time.

Variables	Batches	Ripening time (Days)					SEM	*P value*
		0	3	15	30	45		
pH	CON	5.77^{A}	5.26^{aB}	5.06^{aC}	5.05^{bC}	5.13^{bC}	0.04	0.001
	FOS	5.80^{A}	$5,28^{aB}$	5.09^{aD}	5.19^{aC}	5.22^{aBC}	0.03	0.001
	FOS.BGP1	5.75^{A}	5.21^{aB}	4.76^{bC}	4.77^{cC}	4.74^{cC}	0.05	0.001
	FOS.GG	5.76^{A}	5.00^{bB}	4.65^{cD}	4.71^{cCD}	4.75^{cC}	0.05	0.001
SEM		0.01	0.03	0.03	0.03	0.03		
P value		0.625	0.001	0.001	0.001	0.001		
a_w	CON	0.968^{A}	0.958^{bA}	0.930^{aB}	0.904^{aC}	0.847^{D}	0.06	0.001
	FOS	0.966^{A}	0.963^{aA}	0.923^{bB}	0.889^{cC}	0.829^{D}	0.07	0.001
	FOS.BGP1	0.964^{A}	0.965^{aA}	0.930^{aB}	0.897^{abC}	0.856^{D}	0.06	0.001
	FOS.GG	0.966^{A}	0.964^{aA}	0.934^{aB}	0.893^{bcC}	0.839^{D}	0.06	0.001
SEM		0.01	0.01	0.01	0.02	0.01		
P value		0.449	0.001	0.001	0.001	0.329		
Moisture (%)	CON	58.13^{bA}	55.31^{bB}	41.62^{bC}	34.51^{D}	32.05^{E}	1.41	0.001
	FOS	61.90^{aA}	57.68^{aB}	41.98^{bC}	33.48^{D}	31.17^{E}	1.64	0.001
	FOS.BGP1	62.56^{aA}	57.78^{aB}	44.40^{aC}	33.80^{D}	31.86^{E}	1.63	0.001
	FOS.GG	62.87^{aA}	58.24^{aB}	45.48^{aC}	32.80^{D}	31.07^{E}	1.69	0.001
SEM		0.35	0.34	0.37	0.28	0.32		
P value		0.001	0.001	0.001	0.180	0.498		

Batches: CON: Control without probiotic strains and without FOS; FOS: Low-fat added with FOS 2 $g.100g^{-1}$; FOS.BGP1: Low-fat added with FOS 2 $g.100g^{-1}$ and *Lactobacillus paracasei*; FOS.GG: Low-fat added with FOS 2 $g.100g^{-1}$ and *Lactobacillus rhmanosus* GG. ^{a,b} Mean values in the same column (different formulation in the same ripening time) with different lower case letter presented significant differences$^{A\text{-}E}$ Mean values in the same row (same formulation in different ripening time) with different uppercase letter presented significant differences. SEM: standard error of mean

A similar trend was found for moisture and a_w, since they are factors intrinsically linked. The moisture content of all formulations decreased significantly ($P \leq 0.05$), due to the dehydration effect that occurs in dry fermented meat products (Fonseca *et al.*, 2015). The moisture content decreased from 58.13% for CON and 61.90% for FOS samples till 32.05% and 31.17% for CON and FOS at the end of ripening process, respectively (Table 1). The significant differences ($P \leq 0.05$) between formulations at the beginning could be the result of the different ingredients. It should be noted that FOS is not included in CON composition, and fat reduction is accompanied by an increase of water content at the beginning of ripening time. After 30 and 45 days of ripening, the results for moisture were not significantly different between the formulations, showing that the addition of probiotic strains and the fat reduction had no effect on this parameter. On the other hand, the inclusion of *Lactobacillus* strains did not modify the moisture content (31.86% and 31.07%, for FOS.BGP1 and FOS.GG batches, respectively), reaching similar values than FOS group at the end of ripening process.

Regarding a_w, all formulations ranged from 0.96 to 0.83. The partial substitution of pork back fat by FOS and the adding of probiotic strains showed no effect on the water activity at 0 and 45 days. These results agree with those reported by other authors studying fermented sausages produced with *Lactobacillus plantarum* (Ba *et al.,* 2016; Ba *et al.,* 2018).

Ripening time had a significant effect on the color parameters (Table 3) ($P \leq 0.05$). A decrease in lightness (L*), was observed during ripening time. The greatest decreases were observed in the low-fat formulations, which could be associated with the highest moisture loss occurring over the ripening time. Similar behavior was observed by other authors studying low-fat fermented sausages (Backes *et al.,* 2013; Fonseca *et al.,* 2015).

On the other hand, the addition of prebiotic fiber did not show significant differences on color parameters, founding slightly higher values in CON group (Table 3). The partial substitution of pork back fat by FOS significantly ($P \leq 0.05$) decreased the lightness at 3 and 30 days. At the end of ripening, despite not being significant, lower values of L* were also observed in low-fat formulations. The higher values obtained for CON could be related to the brilliant aspect associated with the presence of fat, leading to lighter sausages (Grigelmo-Miguel, Abadías-Serós, & Martín-Belloso, 1999; Salazar et al., 2009). In addition, the use of FOS as fat replacer results in the formation of a gel that simulates fat. The loss of moisture that takes place during the ripening of low-fat fermented sausages leads to the development of a cloudy viscose solution, causing a loss of L* (Matuszek, 2001; Roberfroid & Slavin, 2000; Rodríguez, Jiménez, Fernández-Bolaños, Guillén, & Heredia, 2006). In this regard, Salazar *et al.* (2009) also reported that the addition of FOS as a fat substitute in low-fat dry fermented sausage had no significant effect on lightness, redness and yellowness. On the other hand, the addition of the probiotic strains did not show any effect on lightness at the end of ripening process, obtaining slightly lower values in FOS group (Table 3).

Table 3. Effect of the partial replacement of pork back fat by FOS and the addition of probiotic strains on the colour parameters of low-fat fermented sausages during ripening time.

Color parameters	Batches	Ripening time (Days)					SEM	*P value*
		0	3	15	30	45		
L*	CON	56.33^{A}	55.38^{aA}	51.65^{B}	51.17^{aB}	50.77^{B}	0.56	0.001
	FOS	55.00^{A}	52.00^{bB}	47.25^{C}	46.01^{bC}	47.89^{C}	0.67	0.001
	FOS.BGP1	57.99^{A}	53.63^{abB}	54.29^{B}	49.26^{aC}	49.73^{C}	0.64	0.001
	FOS.GG	57.55^{A}	54.72^{aA}	55.43^{A}	49.69^{aB}	48.47^{B}	0.65	0.001
SEM		0.49	0.36	0.62	0.56	0.99		
P value		0.134	0.0033	0.829	0.002	0.693		
a*	CON	10.49^{B}	11.04^{bAB}	12.23^{A}	11.52^{AB}	10.47^{B}	0.21	0.040
	FOS	10.59^{B}	12.87^{aA}	12.52^{A}	10.62^{B}	10.01^{B}	0.22	0.001
	FOS.BGP1	10.15^{C}	13.11^{aA}	11.86^{B}	11.43^{B}	10.47^{C}	0.20	0.001
	FOS.GG	10.82^{BC}	12.02^{abA}	11.80^{AB}	11.00^{BC}	10.15^{C}	0.17	0.001
SEM		0.17	0.23	0.18	0.18	0.21		
P value		0.577	0.000	0.489	0.261	0.819		
b*	CON	14.79^{A}	11.88^{bB}	9.47^{CD}	8.79^{aD}	11.03^{BC}	0.38	0.001
	FOS	14.73^{A}	12.90^{aA}	9.58^{B}	7.71^{bC}	10.52^{B}	0.43	0.001
	FOS.BGP1	15.21^{A}	13.30^{aB}	9.35^{D}	7.80^{bE}	11.37^{C}	0.42	0.001
	FOS.GG	15.72^{A}	12.42^{abB}	9.76^{CD}	8.34^{abD}	11.04^{BC}	0.44	0.001
SEM		0.16	0.17	0.14	0.14	0.62		
P value		0.085	0.000	0.758	0.019	0.973		

Batches: CON: Control without probiotic strains and without FOS; FOS: Low-fat added with FOS 2 g.100g^{-1};FOS.BGP1: Low-fat added with FOS 2 g.100g^{-1} and *Lactobacillus paracasei*; FOS.GG: Low-fat added with FOS 2 g.100g^{-1} and *Lactobacillus rhmanosus* GG. ^{a,b} Mean values in the same column (different formulation in the same ripening time) with different lowercase letter presented significant differences. $^{A-E}$ Mean values in the same row (same formulation in different ripening time) with different uppercase letter presented significant differences. SEM: standard error of mean.

The redness value (a*) of all low-fat formulations displayed significant variations throughout the ripening time ($P \leq 0.05$). However, the values obtained at the beginning and at the end of ripening were very similar. In contrast, fat reduction hardly had any influence on redness, which matches the findings reported by Fonseca *et al.* (2015). Only significant differences were observed at the beginning ($P \leq 0.05$), once the fermentation process finished. In this case, the samples with FOS showed higher values (higher than 12.00) than CON (mean values of 11.04). This trend on redness is in line with that obtained by Grigelmo-Miguel *et al.* (1999) who found that replacement of fat with dietary fiber gives place to redder frankfurters. Regarding the addition of the probiotic strains, we did not display any effect on redness at the end of ripening process, obtaining slightly higher values in FOS.BGP1 batch (Table 3).

Yellowness (b*) of all formulations was significantly affected ($P \leq 0.05$) by the ripening time and scarcely affected by the pork back fat substitution. The decreased b* values during ripening (Table 3) had previously been reported in other fermented sausages (Fonseca *et al.*, 2015; Pérez-Alvarez, Sayas-Barberá, Fernández-López, & Aranda-Catalá, 1999). The addition of FOS as fat substitute in low-fat dry fermented sausage did not display any effect on yellowness at the end of ripening (Salazar *et al.*, 2009). In contrast, a significant effect ($P \leq 0.05$) was seen at day 3. The trend for yellower sausages was obtained when dietary fiber was added to Spanish *Salchichón* formulations, similar to the results reported by Grigelmo-Miguel *et al.* (1999) for low fat high dietary fiber frankfurters. Concerning to the addition of the probiotic strains, FOS.BGP1 batch presented higher b* values compared to the other ones, although these differences were not significant (Table 3).

The addition of FOS as fat replacer and probiotic strains had a significant effect ($P \leq 0.05$) on the textural parameters (Table 4). Hardness, gumminess and chewiness increased significantly over the ripening period ($P \leq 0.05$). During fermentation, structural changes due to the decrease in pH cause aggregation of myofibrillar proteins to take place and after fermentation, drying affects the rheological properties (González-Fernández, Santos, Rovira, & Jaime, 2006). In contrast, cohesiveness and springiness decreased significantly during ripening time ($P \leq 0.05$). After 3 days, FOS.GG was the hardest ($P \leq 0.05$) probably due the lowest pH value.

At the end of ripening, the hardness values found for CON were lower than those obtained for the FOS group (Table 4), probably due to the more pronounced moisture loss that occurred in samples with reduced fat content during the dry-curing process (Table 1). Similar behavior was observed in gumminess values, since the FOS samples showed higher values (117.36 N *vs*. 115.78 N, for FOS and CON batches, respectively). In the present study, FOS showed a limited effect which contrasts with the technological properties associated with this fiber and with the results found in other products (Salazar et al., 2009). Its gelling ability usually means softer products and, therefore, a reduction in the force required to compress the sample during the chewing process.

On the other hand, the addition of the probiotic strains increased the hardness values from 300.95 N to 377.49 N, for FOS and FOS.BGP1 batches, respectively. A similar pattern was also noticed by other authors in dry-cured sausages inoculated with different starter cultures (Domínguez, Munekata, Agregán, & Lorenzo, 2016), whereas Casquete *et al.* (2011) found a decrease in hardness values as a result of the effect of starter cultures on protein hydrolysis (Benito *et al.*, 2007). The *Lactobacillus rhmanosus* GG strain presents a more diversified and efficient proteolytic system than bacteria commonly used as starter cultures (Galli *et al.*, 2019), which may justify the lowest hardness value when compared to the *Lactobacillus paracasei* at the end of ripening time.

Table 4. Effect of the partial replacement of pork back fat by FOS and the addition of probiotic strains on the texture profile (TPA) of low-fat fermented sausages during ripening time

Texture parameters	Batches	Ripening time (Days)					SEM	*P value*
		0	3	15	30	45		
Hardness (N)	CON	9.77^{C}	26.38^{bC}	178.41^{bB}	206.98^{cA}	288.59^{bA}	16.47	0.001
	FOS	8.33^{C}	22.34^{bC}	161.05^{bB}	242.97^{bcA}	300.95^{bA}	16.46	0.001
	FOS.BGP1	8.82^{D}	24.92^{bD}	223.52^{aC}	292.87^{aB}	377.49^{aA}	19.69	0.001
	FOS.GG	9.49^{E}	75.43^{aD}	188.22^{bC}	303.28^{abB}	344.69^{abA}	17.05	0.001
SEM		0.23	3.42	6.98	10.01	12.66		
P value		0.107	0.000	0.006	0.007	0.044		
Springiness (mm)	CON	0.749^{aA}	0.531^{bCD}	0.607^{B}	0.550^{C}	0.496^{bcD}	0.10	0.001
	FOS	0.577^{bA}	0.532^{bB}	0.598^{A}	0.526^{B}	0.477^{cC}	0.07	0.001
	FOS.BGP1	0.631^{bA}	0.492^{bC}	0.620^{A}	0.559^{B}	0.526^{aBC}	0.07	0.001
	FOS.GG	0.604^{bAB}	0.641^{aA}	0.635^{A}	0.571^{B}	0.521^{abC}	0.06	0.001
SEM		0.02	0.07	0.04	0.04	0.04		
P value		0.000	0.000	0.108	0.058	0.001		
Cohesiveness	CON	0.518^{aA}	0.374^{bC}	0.416^{bB}	0.411^{cB}	0.404^{abBC}	0.01	0.001
	FOS	0.440^{bA}	0.321^{Cd}	0.433^{bAB}	0.420^{bcB}	0.387^{bC}	0.01	0.001
	FOS.BGP1	0.490^{aA}	0.311^{cC}	0.442^{abB}	0.432^{bB}	0.418^{aB}	0.01	0.001
	FOS.GG	0.433^{bB}	0.463^{aA}	0.469^{aA}	0.451^{aAB}	0.396^{bC}	0.01	0.001
SEM		0.01	0.01	0.01	0.01	0.01		
P value		0.000	0.000	0.008	0.000	0.003		
Gumminess (N)	CON	5.09^{aC}	9.71^{bC}	74.27^{bB}	83.91^{bA}	115.78^{bA}	6.65	0.001
	FOS	3.64^{bC}	7.15^{bC}	73.07^{bB}	99.32^{bA}	117.36^{bA}	6.68	0.001
	FOS.BGP1	4.31^{bD}	7.74^{bD}	98.95^{aC}	124.37^{aB}	158.85^{aA}	8.49	0.001
	FOS.GG	4.13^{bD}	35.08^{aC}	88.24^{abB}	133.51^{aA}	136.72^{abA}	7.07	0.001
SEM		0.14	1.79	3.00	4.70	5.70		
P value		0.000	0.000	0.003	0.000	0.015		
Chewiness (N·mm)	CON	37.62^{aC}	49.70^{bC}	440.97^{bB}	491.93^{bA}	563.76^{bA}	34.42	0.001
	FOS	21.09^{bD}	37.30^{bD}	420.41^{bC}	522.65^{bA}	541.00^{bB}	32.56	0.001
	FOS.BGP1	27.14^{bC}	37.75^{bC}	600.41^{aB}	688.55^{aA}	819.08^{aA}	45.64	0.001
	FOS.GG	24.96^{bE}	220.38^{aD}	549.61^{zC}	741.93^{aA}	700.38^{aB}	38.24	0.001
SEM		1.43	11.73	17.97	25.34	29.44		
P value		0.000	0.000	0.000	0.000	0.000		

Batches: CON: Control without probiotic strains and without FOS; FOS: Low-fat added with FOS 2 g.100g^{-1}; FOS.BGP1: Low-fat added with FOS 2 g.100g^{-1} and *Lactobacillus paracasei*; FOS.GG: Low-fat added with FOS 2 g.100g^{-1} and *Lactobacillus rhmanosus* GG.^{a,b} Mean values in the same column (different formulation in the same ripening time) with different lowercase letter presented significant differences $^{A-E}$ Mean values in the same row (same formulation in different ripening time) with different uppercase letter presented significant differences. SEM: standard error of mean.

3.2. *Lipid oxidation during ripening*

The addition of FOS as fat replacer and probiotics strains did not display a significant effect on the lipid oxidation (Fig. 1). The ripening time showed no effect on the FOS, FOS.BGP1 and FOS.GG samples. After 3 days, the TBARS values increased for all formulations, except for FOS.GG which decreased. This increase could be due to the oxidation of polyunsaturated fatty acids of the phospholipids fraction that occur during fermentation (Visessanguan, Benjakul, Riebroy, Yarchai, & Tapingkae, 2006).

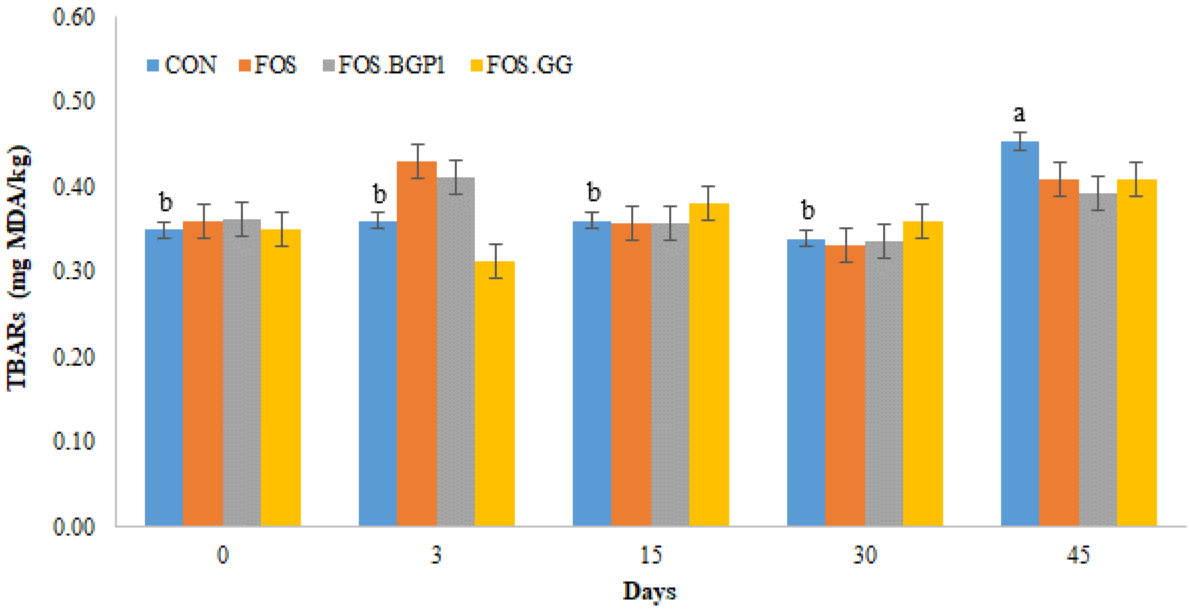

Figure 1. Effect of the partial replacement of pork back fat by FOS and the addition of probiotic strains on TBARS values of low-fat fermented sausages during ripening time.

Batches: CON: Control without probiotic strains and without FOS; FOS: Low-fat added with FOS 2 $g.100g^{-1}$; FOS.BGP1: Low-fat added with FOS 2 $g.100g^{-1}$ and *Lactobacillus paracasei*; FOS.GG: Low-fat added with FOS 2 $g.100g^{-1}$ and *Lactobacillus rhmanosus* GG.

^{a,b} Mean values in the same batch with different letter presented significant differences during the ripening process.

At the end of process, CON samples showed higher TBARS values compared to FOS group (0.45 and 0.41 mg MDA/kg, for CON and FOS treatments, respectively). Our TBARS values were lower than those reported for *Salchichón* (Fonseca *et al.*, 2015; Lorenzo & Franco, 2012). This result was expected due to the low-fat content of FOS group. This finding agrees with data reported by other authors (Fonseca *et al.*, 2015; Gómez, & Lorenzo, 2013) who observed lower TBARS values in samples with low-fat content.

Regarding the addition of the probiotic strains, samples with *Lactobacillus paracasei* presented the lowest values (0.39, 0.41 and 0.41 mg MDA/kg sample, for FOS.BGP1, FOS.GG and FOS batches, respectively), probably due to the antioxidant properties associated with the *Lactobacillus* species (Yadav, 2017). Despite these increases in TBARS values during processing, all formulations were below 2.0 mg MDA/kg, which is accepted as the deterioration level (Greene & Cumuze, 1982).

3.3. Free amino acid content during ripening

Free amino acids are of great importance for their contribution to the taste and also to the volatile compounds derived from the degradation reactions in which they are involved (Bermúdez, Franco, Carballo, Sentandreu, & Lorenzo, 2014). Statistical analysis showed that free amino acid (FAA) content in the low-fat Spanish *Salchichón* was significantly ($P \leq 0.05$) affected by the ripening time (Table 5). An increase in the total FAA was observed from the start to the end of dry-ripening. Glutamic acid and arginine were the most abundant FAA, which represented 27% and 21% of the total FAA, respectively. Alanine, taurine and leucine were the next in importance, with 7.7, 7.1 and 4.8%, respectively. These values were lower than those found by other authors in dry-fermented sausages (Domínguez *et al.*, 2016; Gallego, Mora, Escudero, & Toldrá, 2018).

Regarding the effect of the addition of FOS, only some FAA showed significant differences between formulations. At the end of ripening, only aspartic acid, histidine, tyrosine and lysine showed that FOS had a significant ($P \leq 0.05$) effect on FAA. The main process involved in FAA release is the proteolysis of meat proteins by endogenous enzymes and aminopeptidase activity (Lorenzo & Franco, 2012). In the present study, a greater release of amino acids was observed in the CON fermented sausages. In this regard, higher contents of aspartic acid (13.09 *vs*. 7.99 mg/100 g), histidine (22.49 *vs*. 15.35 mg/100 g) and lysine (28.53 *vs*. 18.97 mg/100 g) were observed in CON batch compared to FOS treatment. On the contrary, tyrosine presented lower values in CON group (5.95 *vs*. 9.26 mg/100 g, for CON and FOS treatments, respectively).

Table 5. Effect of the partial replacement of pork back fat by FOS and the addition of probiotic strains on the evolution of free amino acids of low-fat fermented sausages during ripening time (mg/100g of dry extract).

FAA	Batches	Ripening time (Days)					SEM	*P value*
		0	3	15	30	45		
Aspartic acid	CON	7.69^{BC}	5.03^{bC}	11.18^{bAB}	10.14^{AB}	13.09^{aA}	0.63	0.001
	FOS	7.27^{A}	4.46^{bB}	9.66^{bA}	7.72^{A}	7.99^{bA}	0.48	0.013
	FOS.BGP1	7.78^{BC}	5.94^{bC}	14.41^{abA}	9.83^{B}	9.57^{abB}	0.54	0.001
	FOS.GG	8.23^{C}	8.25^{aC}	18.58^{aA}	10.51^{BC}	12.94^{aB}	0.80	0.001
SEM		0.41	0.31	0.90	0.68	0.57		
P value		0.865	0.000	0.003	0.408	0.006		
Serine	CON	11.07^{C}	14.12^{C}	30.75^{A}	24.75^{B}	34.55^{A}	1.42	0.001
	FOS	11.05^{B}	14.69^{B}	27.73^{A}	25.43^{A}	27.99^{A}	1.39	0.001
	FOS.BGP1	12.26^{C}	16.96^{C}	36.78^{A}	25.05^{B}	27.92^{B}	1.39	0.001
	FOS.GG	11.10^{C}	18.03^{BC}	35.89^{A}	22.39^{B}	31.32^{A}	1.62	0.001
SEM		0.49	0.69	1.62	1.15	1.26		
P value		0.784	0.164	0.152	0.796	0.195		
Glutamic acid	CON	110.20^{cB}	126.41^{B}	207.44^{A}	145.75^{B}	202.59^{A}	7.48	0.001
	FOS	128.73^{bcB}	135.61^{B}	203.54^{A}	170.45^{AB}	188.39^{A}	8.41	0.013
	FOS.BGP1	137.85^{abC}	146.85^{C}	25283^{A}	158.69^{BC}	187.43^{B}	7.85	0.001
	FOS.GG	141.98^{aB}	159.58^{B}	260.42^{A}	149.95^{B}	217.19^{A}	10.03	0.001
SEM		4.33	5.98	11.74	7.82	7.74		
P value		0.041	0.234	0.187	0.704	0.495		
Glycina	CON	8.19^{C}	10.15^{C}	18.63^{B}	16.45^{B}	22.77^{A}	0.85	0.001
	FOS	8.49^{B}	9.81^{B}	17.21^{A}	16.95^{A}	19.38^{A}	0.87	0.001
	FOS.BGP1	9.00^{C}	11.01^{C}	20.78^{A}	16.10^{B}	18.28^{AB}	0.74	0.001
	FOS.GG	8.80^{C}	12.13^{BC}	21.80^{A}	14.87^{B}	20.92^{A}	0.95	0.001
SEM		0.31	0.39	0.99	0.63	0.82		
P value		0.806	0.143	0.358	0.697	0.236		
Histidine	CON	19.47^{B}	20.23^{abB}	31.84^{A}	21.77^{B}	22.49^{aB}	1.25	0.013
	FOS	22.21^{AB}	18.40^{bB}	27.90^{A}	18.42^{B}	15.35^{bB}	1.19	0.001
	FOS.BGP1	23.22^{AB}	17.93^{bBC}	29.09^{A}	16.64^{BC}	12.52^{bC}	1.31	0.001
	FOS.GG	21.82^{B}	24.51^{aB}	38.87^{A}	21.85^{B}	21.95^{aB}	1.28	0.001
SEM		0.76	0.83	1.89	1.38	1.14		
P value		0.442	0.020	0.168	0.471	0.001		
Taurine	CON	32.57^{bB}	33.14^{bB}	48.41^{A}	37.50^{B}	50.99^{A}	1.81	0.001
	FOS	40.24^{aBC}	36.95^{abC}	52.91^{AB}	49.22^{ABC}	53.70^{A}	2.06	0.022
	FOS.BGP1	43.33^{aBC}	38.92^{abC}	61.02^{A}	45.97^{BC}	51.41^{B}	1.68	0.000
	FOS.GG	42.29^{aB}	44.73^{aB}	67.31^{A}	46.56^{B}	54.55^{AB}	2.40	0.003
SEM		1.28	1.42	3.16	1.81	2.00		
P value		0.009	0.029	0.149	0.114	0.917		
Arginine	CON	234.18^{bB}	241.71^{bB}	320.89^{bA}	222.69^{B}	195.16^{B}	10.57	0.002
	FOS	286.64^{aA}	248.27^{abAB}	309.98^{bA}	211.10^{B}	136.63^{C}	13.03	0.001
	FOS.BGP1	306.24^{aB}	262.07^{abB}	390.21^{abA}	199.53^{C}	127.00^{D}	14.14	0.001
	FOS.GG	305.79^{aB}	307.22^{aB}	425.64^{aA}	243.11^{B}	157.61^{C}	17.21	0.001
SEM		8.18	10.89	17.97	11.66	10.31		
P value		0.002	0.000	0.000	0.606	0.089		
Threonine	CON	17.01^{bC}	21.57^{C}	35.01^{A}	27.17^{B}	35.27^{A}	1.25	0.001
	FOS	20.38^{aC}	22.04^{BC}	34.45^{A}	29.83^{AB}	29.77^{AB}	1.38	0.003
	FOS.BGP1	22.07^{aB}	22.12^{B}	39.88^{A}	26.91^{B}	26.49^{B}	1.17	0.001
	FOS.GG	21.05^{aB}	24.97^{B}	38.73^{A}	24.55^{B}	28.27^{B}	1.44	0.001
SEM		0.58	0.91	1.73	1.15	1.28		
P value		0.009	0.551	0.624	0.458	0.083		
Alanine	CON	27.27^{C}	31.66^{BC}	52.26^{A}	39.61^{B}	61.26^{A}	2.17	0.001

	FOS	30.28^{B}	31.06^{B}	49.66^{A}	45.10^{A}	54.24^{A}	2.27	0.001
	FOS.BGP1	32.99^{B}	33.56^{B}	58.69^{A}	42.61^{B}	52.66^{A}	1.96	0.001
	FOS.GG	31.52^{B}	36.84^{B}	63.14^{A}	39.40^{B}	59.70^{A}	2.58	0.001
SEM		0.99	1.36	2.82	1.80	2.27		
P value		0.217	0.445	0.323	0.652	0.485		
Proline	CON	7.92^{C}	10.09^{C}	17.63^{A}	13.97^{bB}	20.81^{A}	0.81	0.001
	FOS	9.23^{B}	10.97^{B}	19.07^{A}	18.91^{aA}	23.17^{A}	1.03	0.001
	FOS.BGP1	10.18^{C}	12.45^{C}	22.34^{A}	16.27^{abB}	21.99^{A}	0.85	0.001
	FOS.GG	9.72^{B}	12.57^{B}	18.69^{A}	12.43^{bB}	20.23^{A}	0.85	0.001
SEM		0.36	0.46	1.09	0.75	0.86		
P value		0.127	0.161	0.467	0.009	0.642		
Tyrosine	CON	7.17	4.49^{c}	4.76^{c}	5.67^{c}	5.95^{c}	0.53	0.536
	FOS	7.10	8.01^{b}	9.00^{b}	8.93^{b}	9.26^{bc}	0.42	0.476
	FOS.BGP1	7.56^{C}	9.00^{bBC}	12.17^{bAB}	10.21^{bB}	12.95^{bA}	0.39	0.001
	FOS.GG	7.87^{C}	13.88^{aB}	17.78^{aAB}	14.79^{aB}	19.26^{aA}	0.81	0.001
SEM		0.28	0.70	0.95	0.66	0.99		
P value		0.770	0.000	0.000	0.000	0.000		
Valine	CON	6.55^{C}	9.95^{C}	19.68^{B}	16.93^{B}	28.84^{A}	1.19	0.001
	FOS	6.56^{C}	10.81^{C}	18.61^{B}	19.59^{B}	26.20^{A}	1.20	0.001
	FOS.BGP1	6.96^{C}	10.87^{C}	20.61^{B}	17.48^{B}	24.38^{A}	1.00	0.001
	FOS.GG	6.83^{D}	10.68^{CD}	20.57^{B}	14.24^{C}	25.35^{A}	1.10	0.001
SEM		0.33	0.39	0.99	0.74	1.05		
P value		0.965	0.719	0.889	0.077	0.487		
Methionine	CON	4.55^{C}	5.65^{C}	11.29^{B}	10.21^{B}	15.35^{A}	0.61	0.001
	FOS	4.34^{B}	5.27^{B}	10.68^{A}	11.10^{A}	13.67^{A}	0.62	0.001
	FOS.BGP1	4.63^{C}	6.21^{C}	13.47^{A}	11.21^{B}	14.34^{A}	0.60	0.001
	FOS.GG	4.71^{C}	7.06^{C}	14.65^{A}	10.60^{B}	16.58^{A}	0.73	0.001
SEM		0.20	0.25	0.65	0.42	0.53		
P value		0.932	0.059	0.106	0.826	0.233		
Lysine	CON	12.42^{C}	15.79^{C}	33.85^{A}	23.57^{B}	28.53^{aAB}	1.42	0.000
	FOS	13.50^{C}	14.67^{BC}	28.71^{A}	21.69^{AB}	18.97^{bBC}	1.30	0.001
	FOS.BGP1	14.47^{C}	14.73^{C}	37.70^{A}	21.72^{B}	20.21^{bBC}	1.45	0.000
	FOS.GG	14.60^{C}	18.17^{BC}	40.88^{A}	22.15^{BC}	25.99^{abB}	1.56	0.001
SEM		0.47	0.69	1.87	1.53	1.29		
P value		0.332	0.249	0.113	0.971	0.020		
Isoleucine	CON	4.30^{C}	6.34^{C}	12.86^{B}	11.46^{B}	19.22^{A}	0.79	0.001
	FOS	4.14^{C}	6.08^{C}	11.99^{B}	13.03^{B}	17.50^{A}	0.80	0.001
	FOS.BGP1	4.21^{D}	6.78^{C}	13.25^{B}	11.81^{B}	16.49^{A}	0.69	0.001
	FOS.GG	4.23^{D}	6.53^{D}	13.74^{B}	9.75^{C}	17.46^{A}	0.77	0.001
SEM		0.22	0.23	0.65	0.47	0.70		
P value		0.995	0.768	0.814	0.100	0.590		
Leucine	CON	8.71^{C}	13.41^{C}	26.57^{B}	23.42^{B}	38.46^{A}	1.58	0.001
	FOS	8.61^{C}	12.97^{C}	24.36^{B}	25.79^{B}	34.23^{A}	1.55	0.001
	FOS.BGP1	8.95^{D}	14.82^{C}	28.79^{B}	25.03^{B}	34.08^{A}	1.42	0.001
	FOS.GG	8.83^{C}	14.35^{BC}	29.75^{A}	20.25^{B}	35.52^{A}	1.58	0.001
SEM		0.45	0.49	1.38	0.96	1.35		
P value		0.994	0.524	0.528	0.182	0.651		
Phenylalanine	CON	5.60^{C}	8.34^{C}	15.45^{B}	15.21^{B}	21.94^{A}	0.87	0.001
	FOS	5.56^{C}	7.90^{C}	14.16^{B}	15.99^{AB}	19.21^{A}	0.84	0.001
	FOS.BGP1	5.82^{D}	8.93^{C}	16.28^{AB}	14.90^{B}	18.12^{A}	0.71	0.001
	FOS.GG	5.64^{D}	9.22^{C}	16.68^{A}	12.83^{B}	19.14^{A}	0.80	0.001
SEM		0.22	0.27	0.74	0.48	0.71		
P value		0.409	0.316	0.656	0.109	0.265		

Batches: CON: Control without probiotic strains and without FOS; FOS: Low-fat added with FOS 2 $g.100g^{-1}$; FOS.BGP1: Low-fat added with FOS 2 $g.100g^{-1}$ and *Lactobacillus paracasei*; FOS.GG: Low-fat added with FOS 2 $g.100g^{-1}$ and *Lactobacillus rhmanosus* GG. ^{a,b} Mean values in the same column (different formulation in the same ripening time) with different lowercase letter presented significant differences. [A-E] Mean values in the same row (same formulation in different ripening time) with different uppercase letter presented significant differences. SEM: standard error of mean.

On the other hand, the addition of the probiotic strains also displayed significant (P≤0.05) differences for aspartic acid, histidine, tyrosine and lysine at the end of ripening process among treatments. In our study, the highest values for these FAA were found in FOS.GG batch: aspartic acid (7.99, 9.57 and 12.94 mg/100 g, for FOS, FOS.BGP1 and FOS.GG treatments, respectively), histidine (15.35, 12.52 and 21.95 mg/100 g), for FOS, FOS.BGP1 and FOS.GG groups, respectively), tyrosine (9.26, 12.95, and 19.26 mg/100 g, for FOS, FOS.BGP1 and FOS.GG batches, respectively) and lysine (18.97, 20.21 and 25.99 mg/100 g, for FOS, FOS.BGP1 and FOS.GG treatments, respectively). This outcome could be related to the proteolytic activity linked to the microbial enzymes of the different starter cultures, where the proteolysis would be reduced avoiding unpleasant aromas (Berardo, Claeys, Vossen, Leroy, & De Smet, 2015). This result is in agreement with the results found by other authors for fermented sausages (Dominguez *et al.*, 2016; Du *et al.*, 2019) who noticed that the inclusion of starter cultures increased the proteolysis. However, Candogan, Wardlaw and Acton (2009) and Casaburi *et al.* (2007) did not observe differences in the amino acid release between different starter cultures. On the contrary, Aro *et al.* (2010) reported that the association of *S. xylosus* with other cultures (*P. pentosaceus* and *L. sakei*) showed reduction on the FAA content.

3.4. Microbiological analysis

The microbial counts for LAB, yeast, *Enterobacteriaceae* and mesophilic aerobes were significantly different (P≤0.05) between formulations in the beginning and during the ripening time (Fig 2). At the beginning of the ripening, LAB and *Enterobacteriaceae* displayed significant (P≤0.05) differences between CON and FOS formulations, finding the highest values in CON group for both microorganisms. On the other hand, the addition of the probiotic strains only showed significant (P≤0.05) differences in LAB counts (Fig 2A) at the beginning of the process. These differences in LAB were related to the addition of *Lactobacillus* starters in FOS.BGP1 and FOS.GG formulations, presenting the highest counts in FOS.GG group (6.45, 6.97 and 7.97 log CFU/g, for FOS, FOS.BGP1 and FOS.GG treatments, respectively). This result agrees with data found in previous studies (Lorenzo, Gómez, & Fonseca, 2014; Zhao *et al.*, 2011) who observed higher LAB counts in inoculated samples compared to the control group.

During the ripening, two main steps were observed in all the microbial determinations: an increase after the fermentation and progressive stabilization until the end of the process. After 45 days of ripening, the addition of FOS did not show significant differences between groups (8.19 and 8.33 log CFU/g, for CON and FOS batches, respectively). However, LAB counts were significantly ($P \leq 0.05$) lower for the FOS samples than for the FOS.BGP1 and FOS.GG groups, which are in agreement with the outcomes found in other sausages that contain short-chain fructooligosaccharides in their composition (Salazar, García, & Selgas, 2009). The use of the probiotic cultures in the low-fat Spanish *Salchichón* is beneficial since they are associated with the inhibition of pathogenic and spoilage bacteria, improving the stability and safety of fermented sausages (Du *et al.*, 2019). This positive effect was reflected in the present study since the *Enterobacteriaceae* counts were significantly ($P \leq 0.05$) lower in the formulations that contain *Lactobacillus* (Fig 2C). This lower activity is associated with the formation of lactic and acetic acids as well as possible bacteriocins (Villani *et al.*, 2007).

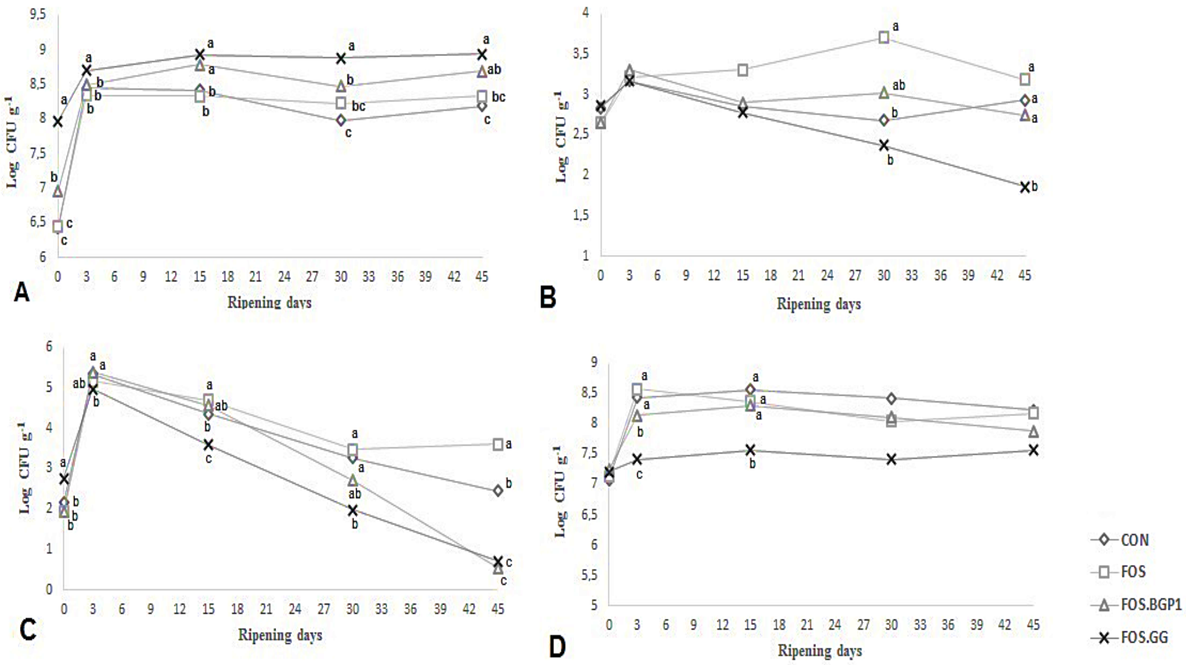

Figure 2. Effect of the addition of FOS and probiotic strains on LAB count (**A**); Yeast (**B**); *Enterobacteriaceae* (**C**) and Mesophilic aerobes (**D**) of low-fat fermented sausages during ripening time.

Batches: CON: Control without probiotic strains and without FOS; FOS: Low-fat added with FOS 2 g.100g^{-1}; FOS.BGP1: Low-fat added with FOS 2 g.100g^{-1} and *Lactobacillus paracasei*; FOS.GG: Low-fat added with FOS 2 g.100g^{-1} and

Lactobacillus rhmanosus GG. [a,b,c] Mean values in the same sampling point with different letter presented significant differences among treatments.

Regarding yeast, unlike other studies, significant differences ($P \leq 0.05$) were found in relation to starter cultures in the last two ripening points (Montanari *et al.,* 2018; Tabanelli *et al.,* 2012). The final counts showed that the lowest values were found in FOS.GG, while FOS presented the highest counts (Fig 2B). However, the addition of FOS did not show significant differences between groups (2.93 and 3.19 log CFU/g, for CON and FOS batches, respectively). This finding also confirms the important action of probiotic cultures in the inhibition of not desirable microorganisms in fermented meat products. On the other hand, it is well known that *Debaryomyces hansenii* is a yeast that positively influences many of the different meat sausages all over the world (Andrade, Córdoba, Casado, Córdoba, & Rodríguez, 2010; Cano-García, Belloch, & Flores, 2014).

The *Enterobacteriaceae* counts showed a significant decrease over the ripening time, especially from day 3 when the counts fell sharply until the end of dry-ripening. At the end of ripening process, samples that contained probiotic strains in their composition showed ($P \leq 0.05$) lower *Enterobacteriacea* counts (Fig 2C) than FOS samples. A similar trend was observed in other sausages inoculated with different starter cultures (Du *et al.,* 2019). The counts found in this study for sausages inoculated with the starter culture and FOS.BGP1 or FOS.GG, were lower than 1 CFU/g, which demonstrated their role in increasing the safety of low-fat Spanish *Salchichón*. On the other hand, the inclusion of FOS increased the *Enterobacteriacea* counts (2.46 and 3.60 log CFU/g, for CON and FOS groups, respectively).

Concerning to mesophilic aerobes, a significant increase was observed after 3 days of ripening time reaching the highest counts in CON and FOS groups (Fig 2D). After 15 days of processing, FOS.GG batch showed the lowest mesophilic values compared to the other ones ($P \leq 0.05$). From 30 days to the end of ripening process, the mesophilic count presented similar levels for all treatments without significant differences among batches. This outcome agrees with data reported by Lorenzo and Franco (2012) who noticed that mesophilic counts increased significantly ($P < 0.001$) during the first 28 days of ripening and then remained stable until the end of process.

3.5. Sensory analysis

Based on the results from the ranking test for the low-fat Spanish *Salchichón* (Table 6), the addition of probiotic strains and the partial reduction on fat content using FOS show no effect on the appearance, texture, flavor and overall acceptance ($P>0.05$). Regarding the inclusion of FOS, the CON group showed higher scores than FOS batch for all attributes studied. On the other hand, the inclusion of *Lactobacillus rhmanosus* improved all sensory parameters, mainly texture (35, 42 and 42, for FOS, FOS.BGP1 and FOS.GG treatments, respectively) and overall acceptability (39, 34 and 44, for FOS, FOS.BGP1 and FOS.GG batches, respectively). These findings are in agreement with those reported by Lorenzo Gómez, Purriños and Fonseca (2016b) who found significant differences among non-inoculated and inoculated dry-cured fermented sausages evaluated by trained judges.

Table 6. Sum of orders according to the preference of consumers (n = 30) in the sensory analysis of Spanish *Salchichón* with probiotics for each attribute

Attributes	Batches			
	CON	FOS	FOS.BGP1	FOS.GG
Appearance	50	35	36	39
Texture	41	35	42	42
Flavor	42	41	34	43
Overall acceptability	43	39	34	44

Batches: CON: Control without probiotic strains and without FOS; FOS: Low-fat added with FOS 2 $g.100g^{-1}$; FOS.BGP1: Low-fat added with FOS 2 $g.100g^{-1}$ and *Lactobacillus paracasei*; FOS.GG: Low-fat added with FOS 2 $g.100g^{-1}$ and *Lactobacillus rhmanosus* GG.

According to Essid and Hassouna (2013), hardness could arise from microbiological and physicochemical processes such as enhanced acidification and proteolysis as a result of *L. sakei, S. xylosus* and *S. carnosus* activities. On the other hand, flavor attributes are linked to compounds (fatty acids, amino acids, aldehydes, esters, etc.) released by microbial and endogenous proteases and lipases throughout ripening of sausages (Essid, & Hassouna, 2013). In this regard, Lorenzo *et al.* (2016b) concluded that the proteolytic and lipolytic activities of the commercial starter cultures used are mainly responsible for the differences in flavor intensity and cured flavor among batches. Our results concluded that the panelists were not able to differentiate the Spanish *Salchichón* formulations for those sensory attributes.

4. CONCLUSION

The results of the present study confirmed that a fermented sausage with fat content reduced using FOS can be developed to produce a new meat product with similar texture, color and sensory characteristics as typical Spanish *Salchichón*. In this regard, the inclusion of FOS could be considered a good strategy to obtain a healthier sausage due to the reduction of fat content by 29% and the recognized prebiotic effects of FOS. In addition, the inclusion of probiotic strains had positive effects on fermented sausages, since they also contribute to the stability and safety of low-fat Spanish *Salchichón*.

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CAPÍTULO 5

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Volatile profile of fermented sausages with commercial probiotic strains and fructooligosaccharides.

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ABSTRACT

The effect of the partial substitution of pork back fat by fructooligosaccharides (FOS) and the probiotic strains *Lactobacillus paracasei* and *Lactobacillus rhmanosus* on the generation of volatile organic compounds in fermented sausages was investigated. The results obtained showed that these factors significantly affected the total content of organic volatile compounds (7484, 8114, 8372 and 10737 AU x 104/g for FOS.GG, CON, FOS.BGP1 and FOS samples, respectively). A total of 59 volatile components, mainly hydrocarbons, ketones and esters were isolated. The reduction of fat content by including FOS in the formulation results in positive effects and a greater stability of the volatile profile of the fermented sausages, increasing ester compounds and reducing the undesirable notes of hexanal (probiotic samples showed values less than 2 AU x 10^4/g). Moreover, there was a symbiotic effect when the aforementioned prebiotic fiber was combined with probiotic *Lactobacillus* strains.

Keywords Meat product · Prebiotic · FOS · Lactic acid bacteria · Aroma · Volatile compounds

1. INTRODUCTION

Both lifestyle and the consumption of some foods have been associated with the development of several diseases increasing the concern of consumers for health. Recently, different strategies have been tested to develop healthy meat products (Jiménez-Colmenero et al. 2006). Functional foods is one of these research areas, resulting in beneficial effects on human health in addition to their nutritious function (Zhang et al. 2010).

Prebiotics are non-digestible substances that serve as substrate to the microorganisms of the human gastrointestinal tract improving human health (De Vrese and Schrezenmeir 2008). Fructooligosaccharides (FOS) are prebiotic foods and, recently, they have been used in functional food research. FOS are oligosaccharides that occur naturally in traditional medicinal plants (Sridevi et al. 2014) and are classified as Generally Recognize as Safe (GRAS), which allows their use in food products as safe additives (FDA 2017). These compounds present a high resistance to digestion and absorption by the gastrointestinal tract resulting in a reduced caloric content (Ruiz-Aceituno et al. 2018). They have a beneficial effect on specific bacteria, stimulating the growth of non-pathogenic intestinal microflora, decreasing the growth of potentially pathogenic strains and enhancing the immune system (Gibson et al. 2017). Besides these effects, it is also worth mentioning the reduction of cholesterol levels and blood pressure, as well as their potential anti-cancer properties (Jiménez-Colmenero et al. 2001). When this prebiotic carbohydrate is administered in combination with strains of probiotic microorganisms, they can act synergically in the human intestines where they ensure the viability of these beneficial microorganisms (Öztürk and Serdaroğlu 2017).

Consequently, the partial substitution of pork fat by functional ingredients in meat products, such as prebiotic dietary fiber, has been the objective of several studies. In fact, the positive effect of partial substitution of pork back fat by prebiotic carbohydrates has been reported in previous studies where FOS is shown to improve the technological parameters, texture properties and sensory characteristics (Salazar et al. 2009; Bis-Souza et al. 2018). A large number of research projects have been carried out in order to develop functional meat products, including the use of potential probiotic strains in fermented sausages (Ba et al. 2018). Traditional fermented sausages, made exclusively of pork lean and back fat, salt and spice (Fonseca et al. 2015), could be a good matrix in which to develop these healthy products. This type of dry-fermented sausage is traditionally made without adding a starter culture. However, the use of a starter culture could guarantee a more hygienic process, which standardizes sensorial and technological characteristics in a shorter ripening time (Benito et al. 2011). Lactic acid bacteria (LAB) are responsible for the acidification of the product during fermentation, resulting in improvements of flavor, taste and the biological safety of the products (Ba et al. 2018). The characteristic aroma of these products is conferred by many different non-volatile and volatile compounds, resulting not only from the activity of the aforementioned bacterial groups but also from spices as well as from the activity of endogenous meat enzymes (Ordóñez et al. 1999). Most commercial fermented sausages use a combination of starter cultures to guarantee the typical flavors and aromas of these meat products. Lactobacillus, Staphylococci and/or Micrococci are the genus strains commonly used (Cheng et al. 2018).

However, there is scarcely any information about FOS and the symbiotic effect when a meat product contains both probiotics and prebiotics in their formulation, especially regarding their volatile profile, which determines the typical aroma of this fermented product. The characteristic flavor of these meats is one of the most appreciated attributes for the consumer and one which really affects their acceptance (Gómez et al. 2015; Bosse et al. 2017).

Therefore, the present study aims to evaluate the influence of the addition of fructooligosaccharides and two different probiotic commercial strains on the volatile profile of low-fat fermented sausages.

2. MATERIAL AND METHODS

2.1. Prebiotic and Probiotic materials

The prebiotic fiber used as fat substitute was NutraFlora® P95 soluble prebiotic fiber (short-chain fructooligosaccharide - moisture 4%; total dietary fiber 95% dry basis) (Ingredion, Westchester, USA).

The commercial *starter* culture used was Bactoferm T-SPX (*Pediococcus pentosaceus + Staphylococcus xylosus*, (Chr.Hansen, Hørsholm, Denmark*)*. The commercial probiotics strains used were the Lyofast BGP 1 composed of *Lactobacillus paracasei* (Sacco System, Cadorago, Italy) and *Lactobacillus rhamnosus* GG (Chr.Hansen, Hørsholm, Denmark).

2.2. Fermented sausage manufacture

Four different batches (CON, FOS, FOS.BGP1 and FOS.GG) of low-fat fermented sausages were manufactured in the pilot plant of the Meat Technology Center of Galicia (San Cibrao das Viñas, Ourense, Spain).

A control formulation (denominated CON) without any probiotic strains or fructooligosaccharides added was prepared using lean pork (80 g/100g), pork back fat (15 g/100g) both from Celta pigs, the “542 Salchichón” supplement (Laboratorios Ceylamix, Valencia, Spain), (5 g/100g, composed, in unknown proportions, of sugar, salt, dextrin, spices (black and white pepper and nutmeg), milk protein, monosodium glutamate (E621), phosphates

(E450i and E451i), sodium erythorbate (E316), potassium nitrate (E252) and coloring (E120)) and a commercial starter culture Bactoferm T-SPX (0.025 g/100g). Three other batches of low-fat fermented sausages were produced using lean pork (80 g/100g), pork back fat (10 g/100g), cold water to homogenize (3 g/100g), FOS (2 g/100g) “542 Salchichón” (5 g/100g) supplement, and commercial *starter* culture Bactoferm T-SPX (0.025 g/100g). The probiotic strains (0.01g/kg) *Lactobacillus paracasei* and *Lactobacillus rhmanosus* GG were added to the two formulations denominated FOS.BGP1 and FOS.GG, respectively. The other formulation with no strain added was denominated FOS.

The lean pork was ground using a 10mm diameter mincing plate while the pork back fat was ground through an 8 mm diameter mincing plate. The batches were mixed in a vacuum mincer (Fuerpla, Valencia, Spain) for 2 min and kept at 3-5 ºC for 24 h. Then, the batch was stuffed into natural casing 35 cm long and 60 mm in diameter, so that the final weight of each sausage was around 400 g. The sausages were kept in a fermentation chamber for 2 days at 20 °C and 80% relative humidity and then transferred into a drying-ripening chamber where they were kept for 43 days at 8-12 °C and 75–60% of relative humidity.

Six replicates from each batch were taken after 45 days of ripening. The four formulations were manufactured in two batches with the same ingredients, formulation and technology, first in March and then in April of 2018.

2.3. Chemical composition

In order to characterize the final products, the proximate composition was determined. Moisture, protein, fat and ash contents were quantified according to Lorenzo et al. (2016).

2.4. Volatile compounds

The extraction of the volatile compounds was performed using solid-phase microextraction (SPME), following the method described by Domínguez et al. (2019) with modifications. For headspace SPME (HS-SPME) extraction, 1 g of each sample, after being ground using a commercial grinder, was placed in a 20 mL vial. The conditioning, extraction and injection of the samples were carried out with a PAL RTC 120 auto sampler (CTC Analytics AG, Zwingen, Switzerland). The extractions were carried out at 37 °C for 30 min, after equilibration of the samples for 15 min at the temperature used for extraction, ensuring a homogeneous temperature for sample and headspace. Once sampling was finished, the fibre was transferred to the injection port of the gas chromatograph–mass spectrometer (GC–MS) system. A 7890B gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a 5977B MSD mass selective detector (Agilent Technologies) and a DB-624 capillary column (30 m, 0.25 mm i.d., 1.4 µm film thickness) (J&W Scientific, Folsom, USA) was used for volatile analysis. Compounds were identified by comparing their mass spectra with those contained in the NIST14 (National Institute of Standards and Technology, Gaithersburg, USA) library, and/or by comparing their mass spectra and retention time with authentic standards (Supelco, Bellefonte, USA) and/or by calculation of the retention index relative to a series of standard alkanes (C5-C14), Supelco 44585-U, (for calculating Linear Retention Index, , (Supelco, Bellefonte, USA) and matching them with data reported in the literature. The results are expressed as area units (AU) × 10^4/g of sample.

2.5. Statistical analysis

A total of 48 fermented sausages: six fermented sausage samples for each batch × four batches (CON, FOS, FOS.BGP, FOS.GG**)** × one ripening time (45 days) × two different manufactured batches (March and April 2018) were analyzed for different dependent variables. After that, normal distribution and variance homogeneity were tested (Shapiro and Wilk, 1965).

For the statistical analysis of the results of low-fat fermented sausages, an analysis of variance (ANOVA) with the mixed-model was performed for all variables considered in the study. These parameters were set as dependent variables, while formulation was included in the model as fixed effect and the different manufacture and replicates were considered as random effects. Duncan's method was used to assess the pairwise differences between least square means wherein such differences were considered significant if $P \leq 0.05$. The values were expressed as mean values and standard error of the mean (SEM). All statistical analysis was performed using Statistica 7.0 software.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of low-fat fermented sausage

As expected, the proximate composition of the different low-fat fermented sausages at the end of ripening showed significant differences for fat content (P<0.05), which is the one of the main objectives of this research. A decrease in the value was observed when low-fat treatments were compared with CON (19.70 g/100g vs. mean values of 13.59 g/100g for CON and low-fat fermented sausages, respectively). This difference is explained by the partial substitution of pork back fat in the low-fat formulations by fructooligosaccharides (FOS). The addition of probiotic strains showed no effect on the fat composition of the different treatments with FOS since the same amount of pork back fat was used in their formulation. Regarding moisture, protein and ash contents, the results obtained show no significant differences between the treatments (mean values of 31.54 g/100g, 16.65 g/100g and 2.86 g/100g for moisture, protein and ash contents, respectively). In general, these results were similar to those reported by other authors in dry-cured sausages (Dominguez et al. 2016).

3.2. Volatile compounds of low-fat fermented sausage

Table 1 shows the effect of FOS and probiotic strains on the volatile profile of low-fat fermented sausages at the end of ripening (expressed as UA x 10^4/g of dry matter). Statistical analysis showed that total volatile compounds contents were significantly affected (*P*<0.05) by FOS and the *Lactobacillus* strains (7484, 8114, 8372 and 10737 AU x 10^4/g for FOS.GG, CON, FOS.BGP1 and FOS samples, respectively).

Hydrocarbons are the major volatile group identified in the volatile profile of the fermented sausages. Their contents represented between 75% and 85% of total volatile compounds. Then, in order of importance, came ketones, esters, and alcohols and organic acids that presented similar content percentages., while aldehydes were the last group.

Regarding the hydrocarbons, 25 have been identified in fermented sausage, terpenes being predominant in the volatile fraction of this product. This could be related to the relatively large amount of spices included in its formulation (Rivas-Cañedo et al. 2009; Domínguez et al. 2016). The addition of FOS and probiotic strains did not have a significant (*P*>0.05) effect on the total content of these groups of volatile compounds. Despite not being significantly different, the FOS treatment displayed the highest amount (7999 AU x 10^4/g *vs* 7110, 6170 and 6054 AU x 10^4/g for FOS *vs* FOS.BGP1, CON and FOS.GG samples, respectively), which could be due to the great affinity of the starter cultures for FOS, selectively favoring its development. While in the samples that contain the starter cultures, FOS and one or another probiotic species, competition for this substrate could occur which would condition the enzymatic reactions that contribute to the development of aroma and flavor (Hierro et al. 1997).

In addition, the contribution of lactic acid bacteria to flavor could be limited by their carbohydrate catabolism, whereas gram-positive strains such as *Staphylococcus* might be more appropriate in the generation of aromatic compounds specific to fermented sausages (Leroy et al. 2006).

Terpenes have their origin in the spices used in manufacture of low-fat fermented sausages, in particular black and white pepper (Montanari et al. 2018). α-Thujene (1252 AU x 10^4/g), β-Terpinene (1024 AU x 10^4/g), (-)-β-Pinene (985 AU x 10^4/g), o-Cymene (1292 AU x 10^4/g) and D-Limonene (777 AU x 10^4/g) were the major individual hydrocarbons identified. Significant contents of γ-Terpinene, α-Phellandrene, 3-Carene, β-Myrcene and α-Terpinene were also found. These results were also seen with other dry-cured meat products (Rivas-Cañedo et al. 2009). Among the terpenes isolated, only α-Thujene, β-Myrcene, β-Phellandrene and δ-Carene show significant (P<0.05) differences between the different treatments. The incorporation of FOS in the formulation of fermented sausages as fat replacer results in the release of a greater amount of volatile terpenes, while α-Thujene showed similar values in FOS and FOS.BGP1 samples. In contrast, volatile compounds that originate from biochemical changes (microbial metabolism and endogenous reactions) were less common. This is confirmed by the fact that the addition of FOS and *Lactobacillus* strains scarcely affected these compounds.

Ketones were the second largest group isolated from the fermented sausages. The total contents were significantly (P<0.001) affected by the compositions of this product. FOS samples showed significantly higher values than those obtained in the other treatments. The contents found in FOS samples (1700 AU x 10^4/g) was almost double the CON contents (977 AU x 10^4/g) and triple and fivefold the amounts detected in FOS.GG (520 AU x 10^4/g) and FOS.BGP1 (348 AU x 10^4/g), respectively. The lower contents observed in the samples that contain the probiotic strains could be related to the greater presence of microbial strains (Sánchez-Peña et al. 2005).

Acetoin, 2-Butanone and 2,3-Octanedione were the main compounds identified. Acetoin was the major ketone detected. As occurred with the total contents, FOS showed significantly ($P<0.001$) higher contents than the other batches (1685 AU x 10^4/g *vs* 960, 486 and 327 AU x 10^4/g for FOS *vs* CON, FOS.GG and FOS.BGP1 samples, respectively). There are two possible origins of this compound, (i) Maillard reactions (Pérez-Santaescolástica et al. 2018) or microbial carbohydrate metabolism (Petričević et al. 2018). This volatile compound has a very low odor threshold, so it contributes to the typical flavor of dry-cured meat products (Sidira et al. 2015). Another important ketone detected was 2-Butanone. Although there was no significant difference, the contents of FOS.GG were higher than the other three treatments. The oxidation of Free Fatty Acids (FFA) is related to the origin of this compound (Narváez-Rivas et al. 2012), and gives important aroma notes to associated meat products because of its peculiar and intense odour (Pastorelli et al. 2003).

Regarding esters, the *Lactobacillus* strains had a significant ($P<0.05$) effect on the total content of this group of volatile compounds (497 and 345 AU x 10^4/g for FOS.BGP1 and FOS.GG *vs* 87 and 105 AU x 10^4/g for FOS *vs* CON samples, respectively). The composition of the identified esters also differed for the formulations under study. Butanoic and hexanoic acid ethyl esters were the most abundant in the FOS.BGP1 and FOS.GG samples (representing 52% and 60% of the total esters, respectively), while acetic acid ethenyl ester and ethyl acetate were predominant in the CON and FOS samples (65% and 79% of the total esters, respectively). The esterase activity of lactic acid bacteria could be the answer to these differences by promoting the enzymatic esterification of fatty acids and alcohols (Narváez-Rivas et al. 2012). The ethyl esters, the main esters detected in the present study, are related to the flavor of fermented sausages as they can mask rancid odors (Andrade et al. 2010).

Four compounds were isolated in the group of alcohols. The total content of this group showed significant differences between the formulations under study (132, 151, 175 and 177 AU x 10^4/g for CON, FOS, FOS.BGP1 and FOS.GG, respectively; $P<0.01$). Terpinen-4-ol was the most abundant, which coincides with previous studies that affirm that terpinen-4-ol, 4-Thujanol and linalool are the alcohols commonly detected in fermented sausage samples (Domínguez et al. 2019). FOS and the two probiotic-strain formulations showed similar values but significantly higher than those observed in CON. This could be related to the activity of *Lactobacillus*, which may favor the formation of the branched aldehydes associated with the origin of these alcohols (Narváez-Rivas et al. 2012).

FOS along with the two added probiotic strains only had a significant effect on one of the organic acids isolated. However, acetic acid showed the lowest values in this group of volatiles. This result does not agree with the results found for other dry fermented sausages (Montanari et al. 2018). Only sausages inoculated with probiotic strains showed any values for this compound, which would explain its origin as being related to carbohydrate fermentation induced by microorganisms (Andrade et al. 2010). Despite not being significant, the other organic acids detected showed higher values in the FOS samples. The detected organic acids are described as potent odorants, so they contribute to the typical aroma of fermented sausage (Corral et al. 2013; Lorenzo et al. 2014).

Aldehyde content represented less than 1% of the total volatile compounds. This result does not agree with the results observed by other authors, who found that this group of volatiles is one of the most important in fermented sausages (Domínguez et al. 2016). The aldehydes detected in the present study had three main origins. Branched aldehydes (butanal, 3-methyl) are related to proteolysis and amino acid degradation (Purriños et al. 2012): cycloaldehydes are derived from Streker degradation of amino acids (Lorenzo and Carballo 2015); and hexanal is related to the lipid oxidation of fatty acids (Montanari et al. 2018).

The CON samples showed significantly (P<0.001) higher amounts of aldehydes than the probiotic and prebiotic batches (75, 63, 51 and 34 AU x 10^4/g for CON, FOS, FOS.GG and FOS.BGP1, respectively). In the present study, benzeneacetaldehyde, benzaldehyde and butanal, 3-methyl- were the main aldehydes isolated. Significant differences were found for the aforementioned compounds (P<0.001). The first one is the most abundant in CON, benzaldehyde in FOS.GG and butanal, 3-methyl in FOS and FOS.BGP1 samples. Previous studies described that *Lactobacillus* strains has protein degradation mechanisms with the capability of producing useful amino acids (Kleerebezem et al. 2003), which are precursors of volatile compounds with a desirable impact on aroma (Toldra and Flores 1998). This could justify the higher content of the cycloaldehydes derived from Strecker degradation of amino acids in samples that contain probiotic strains.

On the other hand, hexanal followed the trend of total aldehydes, with higher content in CON than in FOS and the inoculated samples. In fact, the inoculated samples showed very low values (less than 2 AU x 10^4/g). This result contrasts with the higher content found for this volatile compound in other sausages inoculated with commercial starter cultures (75% of the total aldehydes) (Domínguez et al. 2016).

Finally, sulfur compounds were also detected in low-fat fermented sausages. Significant differences ($P<0.05$) were found in dimethyl disulfide content, the samples inoculated with probiotic strains being the ones which obtained higher values for this volatile compound. The origin of these compounds is associated with the use of spices as ingredients in dry-cured sausages (Sunesen et al. 2001; Muriel et al. 2004).

Table 1. Volatile compounds of low-fat fermented sausage (expressed as UA x 10^4/g dry matter).

Volatile compounds	LRI	R	Bach				*SEM*	*P-value*
			CON	FOS	FOS.BGP1	FOS.GG		
Butanoic acid	929	21.23	77.82	98.08	81.63	84.36	4.37	0.393
Butanoic acid, 3-methyl-	983	23.59	33.10	52.42	39.47	30.89	3.39	0.102
Hexanoic acid	1104	28.79	15.72	16.93	12.50	13.04	0.97	0.313
Acetic acid	684	10.68	0.00^{b}	0.00^{b}	52.77^{a}	66.76^{a}	7.24	0.001
Total Organic Acids			126.63	167.43	186.37	195.05	6.43	0.141
Benzyl alcohol	1149	30.73	57.32	64.83	55.33	68.85	4.04	0.619
4-Thujanol	1151	30.82	10.27^{b}	17.27^{a}	10.39^{b}	9.24^{b}	0.62	0.001
Linalool	1171	31.68	8.95	14.91	11.05	11.38	1.01	0.209
Terpinen-4-ol	1237	34.53	55.74^{b}	79.78^{a}	75.22^{a}	81.81^{a}	2.38	0.001
Total Alcohols			131.55^{b}	176.61^{a}	151.07^{ab}	175.40^{a}	5.41	0.004
Butanal, 3-methyl-	653	9.31	18.55^{b}	26.09^{a}	18.97^{b}	14.44^{b}	1.14	0.001
Hexanal	881	18.75	8.16^{a}	5.77^{b}	0.00^{c}	1.65^{c}	0.59	0.001
Benzaldehyde	1063	27.03	8.92^{bc}	8.20^{c}	13.18^{b}	22.80^{a}	1.15	0.001

Benzeneacetaldehyde	1142	30.44	34.63^{a}	22.47^{b}	7.22^{c}	7.80^{c}	1.89	0.001
(E)-Hexadec-2-enal	1156	31.04	4.47	4.76	5.05	4.83	0.88	0.997
Total Aldehydes			74.73^{a}	63.23^{ab}	34.33^{c}	50.83^{bc}	3.13	0.001
Acetic acid ethenyl ester	576	6.01	39.62	52.21	31.36	34.02	3.25	0.094
Ethyl Acetate	588	6.53	28.05	16.31	21.77	16.51	2.51	0.296
Propanoic acid, ethyl ester	736	12.91	1.82^{a}	0.00^{b}	5.77^{a}	5.31^{a}	0.87	0.049
Butanoic acid, ethyl ester	861	18.29	9.77^{b}	5.05^{b}	135.15^{a}	104.49^{a}	17.96	0.013
Butanoic acid, 2-methyl-, ethyl ester	918	20.75	7.17^{b}	4.65^{b}	65.72^{a}	46.93^{b}	7.16	0.001
Butanoic acid, 3-methyl-, ethyl ester	922	20.93	25.95	16.48	85.95	58.85	13.81	0.257
Hexanoic acid, ethyl ester	1069	27.29	6.17^{b}	2.69^{b}	123.62^{a}	101.75^{a}	15.71	0.004
Octanoic acid, ethyl ester	1233	34.34	2.69^{b}	0.00^{b}	40.18^{a}	29.18^{ab}	5.36	0.013
Total Esters			104.68^{b}	86.82^{b}	497.13^{a}	345.33^{ab}	52.76	0.018
Octane	825	16.76	20.96	20.14	21.15	13.99	1.43	0.251
Heptane, 3-ethyl-	915	20.61	10.95	27.67	36.88	21.39	2.94	0.160
α-Thujene	984	23.61	992.25^{b}	1356.56^{a}	1454.25^{a}	1202.88^{ab}	57.47	0.015
β-Terpinene	992	23.96	957.45	1256.60	1002.93	878.66	72.54	0.280

Heptane, 3-ethyl-5-methylene-	999	24.24	12.92^{a}	11.21^{a}	5.97^{b}	4.63^{b}	0.88	0.001
Camphene	1011	24.76	21.61	29.63	24.10	23.05	1.88	0.465
β-Phellandrene	1037	25.89	138.25^{b}	271.09^{a}	196.58^{ab}	164.31^{b}	17.69	0.041
Nonane, 3-methylene-	1038	25.94	18.54	20.77	17.60	17.42	0.83	0.469
(-)-β-Pinene	1040	26.02	984.10	1162.44	870.61	923.43	59.89	0.357
Decane	1047	26.33	42.82^{a}	38.07^{a}	25.95^{b}	20.95^{b}	2.38	0.001
β-Myrcene	1049	26.41	201.80^{b}	299.34^{a}	235.76^{ab}	211.23^{b}	13.96	0.047
α-Phellandrene	1065	27.11	368.33	407.35	341.50	395.85	31.54	0.894
3-Carene	1069	27.26	356.13	365.14	282.72	285.89	18.33	0.228
α-Terpinene	1077	27.62	150.03	246.85	236.75	205.67	15.26	0.093
D-Limonene	1087	28.06	664.08	908.09	780.71	755.12	32.61	0.053
o-Cymene	1091	28.23	1048.78	1499.01	1399.78	1221.90	77.57	0.169
γ-Terpinene	1113	29.18	267.36	433.11	415.09	369.90	25.71	0.086
Undecane	1135	30.15	70.92^{a}	69.95^{a}	36.51^{b}	33.84^{b}	4.45	0.001
δ-Carene	1139	30.30	59.17^{b}	101.01^{a}	82.75^{ab}	74.46^{ab}	5.23	0.028
4-Vinyl-o-xylene	1153	30.89	47.06	68.32	59.89	50.80	3.73	0.177

Dodecane	1215	33.60	46.18[a]	49.50[a]	37.97[ab]	28.09[b]	2.59	0.007
1-Decene, 2,4-dimethyl-	1230	34.22	0.00[b]	0.00[b]	19.60[a]	22.85[a]	2.43	0.001
Tridecane	1289	36.79	20.34	21.45	17.62	13.60	1.20	0.085
α-Copaene	1365	40.05	19.39	28.96	28.06	24.80	1.39	0.055
β-Caryophyllene	1404	41.75	91.93	106.62	91.14	97.73	4.49	0.599
Alkanes			128.49	142.90	105.23	84.77	10.57	0.223
Alkenes			6041.61	6335.80	5001.18	5969.63	410.08	0.696
Total Hydrocarbons			6170.12	7998.70	7109.80	6054.40	397.01	0.265
2-Butanone	583	6.29	12.85	13.07	17.97	22.72	1.66	0.118
Acetoin	790	15.23	960.44[b]	1684.77[a]	327.25[c]	485.60[c]	103.33	0.001
2,3-Octanedione	1068	27.23	7.48[b]	4.41[b]	85.63[a]	96.29[a]	13.36	0.014
Total Ketones			977.37[b]	1700.04[a]	348.07[c]	520.44[c]	99.09	0.001
Sulfide, allyl methyl	697	11.21	64.76	79.74	59.48	48.55	4.82	0.147
Disulfide, dimethyl	782	14.89	4.30[b]	0.00[b]	17.72[a]	8.22[ab]	2.11	0.018
Total Sulphur compounds			69.06	79.74	77.20	56.77	5.94	0.399
Oxetane	510	3.16	22.53	31.06	25.17	29.63	2.12	0.463
Spiro[2,4]hepta-4,6-diene	807	15.99	21.31	28.16	32.61	32.93	3.66	0.658

Pyrazine, 2,6-dimethyl-	993	24.00	12.16	17.28	13.32	11.63	0.93	0.122
Pyrazine, trimethyl-	1079	27.71	12.70	22.80	20.94	18.97	1.42	0.062
Eucalyptol	1097	28.49	8.48[b]	12.67[a]	10.73[ab]	10.17[ab]	0.54	0.049
Safrole	1318	38.04	276.19[c]	344.08[a]	291.98[bc]	330.04[ab]	8.95	0.018
Methyleugenol	1396	41.38	7.71[b]	13.63[a]	11.56[a]	13.90[a]	0.58	0.001
Myristicin	1462	44.24	12.74[b]	23.43[a]	20.64[a]	23.69[a]	1.05	0.001
Total Other Compounds			373.82	493.11	426.95	470.96	11.49	0.168
Total volatile compounds			8113.90[b]	10737.02[a]	8371.79[ab]	7484.42[b]	446.03	0.048

CON: Control without probiotic strains and without FOS; FOS: Low-fat with FOS 2 $g.100g^{-1}$ added; FOS.BGP1: Low-fat with FOS 2 $g.100g^{-1}$ and *Lactobacillus paracasei* added; FOS.GG: Low-fat with FOS 2 $g.100g^{-1}$ and *Lactobacillus rhmanosus* GG added.LRI: linear retention index. [a,b,c] Mean values in the same row with different letters presented significant differences; SEM: standard error of the mean.

4. CONCLUSIONS

The results found in the present study confirmed that the prebiotic fiber FOS and *Lactobacillus* strains have a significant and positive effect on fermented sausages, since they contribute to the aroma as well as the stability of the volatile profile of low-fat fermented sausages. The main groups of volatile compounds identified in low-fat fermented sausages are terpenes, related to spices used in the manufacture of this product, and some sulfur compounds. The second ones resulted from the dry-curing process. Hexanal, butanal, 3-methyl, acetoin, ethyl esters, acetic acid, butanoic acid and butanoic acid, 3-methyl- are included in this group. These compounds have a high influence in the aroma of fermented sausages.

The incorporation of FOS in the formulation of low-fat fermented sausages as fat replacer causes the release of a greater amount of volatile terpenes. The probiotic strains *Lacobacillus paracasei* and *Lacobacillus rhmanosus* showed high ester content, which would mask undesirable odors associated with fermented sausages. Moreover, the lower values for hexanal obtained in the FOS and the inoculated samples reflected a positive effect of this symbiotic combination against the rancid flavors usually associated with this compound.

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CONCLUSÃO GERAL

A redução parcial da gordura suína (até 29%) em salame tipo Italiano e a incorporação de inulina ou FOS não alteraram as propriedades físico-químicas, estabilidade oxidativa e microbiológica do produto final, entretanto a alfa-ciclo dextrina não apresentou resultados satisfatórios em relação a cor e aceitação sensorial do produto. A estratégia de utilizar fibras alimentares, principalmente a FOS, como substituto parcial da gordura suína em embutido cárneo fermentado (salame tipo italiano) se mostrou eficiente ao manter propriedades físico-químicas e sensoriais satisfatórias aos consumidores.

As cepas de bactérias ácido láticas potencialmente probióticas (*L. casei* SJRP) inicialmente selecionadas e adicionadas no processamento de embutidos fermentados mantiveram contagens elevadas de células viáveis, o que pode colaborar na obtenção de um produto cárneo fermentado com caraterísticas funcionais desejáveis e acarretar benefícios a saúde do consumidor.

As cepas potencialmente probióticas de *L. casei* SJRP 66 e *L. casei* SJRP 169 apresentaram habilidade para aplicação em produtos cárneos com teor reduzido de gordura adicionado de FOS, já que mantiveram alta contagem de células viáveis no produto final.

Os micro-organismos adicionados alteraram parâmetros importantes, como a textura (aumentaram a dureza), cor (diminuíram a intensidade da cor vermelha) e o conteúdo de compostos voláteis no produto final. A maior atividade proteolítica da cepa comercial *Lactobacillus rhamanosus* GG resultou em uma maior quantidade de amino ácidos livres no *Salchichón* Espanhol com teor reduzido de gordura adicionado de FOS.

Foi possível inovar na produção de embutidos cárneos fermentados, produzindo salame tipo Italiano com reduzido teor de gordura adicionado de fibras prebióticas com apelo mais saudável com propriedades sensoriais e físico-químicas similares aos embutidos fermentados tradicionais.

APÊNDICE A- Ficha sensorial utilizada no Capítulo 2.

Nome: ________________________________ Data: ____/07/17 N° da Amostra______________

Você está recebendo uma amostra de salame tipo italiano. Por favor, prove-a a avalie cada item segundo a escala abaixo:

9- gostei extremamente	Aparência	____________
8- gostei muitíssimo		
7-gostei moderadamente	Cor	____________
6- gostei levemente		
5- indiferente	Sabor	____________
4- desgostei levemente		
3- desgostei moderadamente	Textura	____________
2- desgostei muitíssimo		
1-Desgostei extremamente	Aceitação Global	____________

APÊNDICE B- Termo de Consentimento Livre e Esclarecido – TCLE utilizado no Capítulo 2.

Termo de Consentimento Livre e Esclarecido - TCLE

(Conselho Nacional de Saúde, Resolução 466/2012/Resolução 510/2016)

Você está sendo convidado a participar como voluntário do projeto de pesquisa "Fibras prebióticas como substitutos de gordura em salame tipo italiano utilizando delineamento de mistura" sob responsabilidade da pesquisadora Camila Vespúcio Bis. O estudo será realizado através de análise sensorial de um produto cárneo desenvolvido para avaliação da aceitabilidade pelos mesmos. Os riscos físicos à sua saúde são mínimos, uma vez que os produtos tenham sido elaborados seguindo as Boas Práticas de Fabricação. Serão impedidos de participar indivíduos com patologias relacionadas à ingestão de alimentos, como diabetes, hipertensão, intolerância e/ou alergia à lactose, ao glúten ou algum ingrediente presente nas amostras. Esta exclusão se dará pela possível existência de algum risco à saúde do participante. Você poderá consultar a pesquisadora responsável em qualquer época, pessoalmente ou pelo telefone da instituição, para esclarecimento de qualquer dúvida. Você está livre para, a qualquer momento, deixar de participar da pesquisa. Todas as informações por você fornecidas e os resultados obtidos serão mantidos em sigilo e, estes últimos só serão utilizados para divulgação em reuniões e revistas científicas. Você será informado de todos os resultados obtidos, independentemente do fato destes poderem mudar seu consentimento em participar da pesquisa. Você não terá quaisquer benefícios ou direitos financeiros sobre os eventuais resultados decorrentes da pesquisa. Este estudo é importante porque seus resultados fornecerão informações para o desenvolvimento de um produto cárneo mais saudável com teor reduzido de gordura.

Diante das explicações, se você concorda em participar deste projeto, por favor, informe seus dados abaixo e assine este Termo.

Nome____R.G.____________________

Endereço____Fone____________________

E-mail ______________________________São José do Rio Preto, _____de_________de 2017.

_________________________________ _________________________________

Usuário ou responsável legal   Pesquisador (a) responsável

OBS.: Termo apresenta duas vias, uma destinada ao usuário ou seu representante e a outra ao pesquisador

Nome: Camila Vespúcio Bis	Cargo/Função: Doutoranda
Instituição: Departamento de Engenharia e Tecnologia de Alimentos/ IBILCE/UNESP	
Endereço: Rua Cristóvão Colombo, 2265- Jardim Nazareth- São José do Rio Preto/SP- Telefones (17) 3221.2250	
Projeto submetido ao Comitê de Ética em Pesquisa do IBILCE/UNESP São José do Rio Preto – fone 17-3221.2428/2563 e 3221.2482	

ANEXO A- Direitos de uso do artigo do periódico Meat Science em tese.

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Impact of fructooligosaccharides and probiotic strains on the quality parameters of low-fat Spanish Salchichón

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