UNIVERSIDADE ESTADUAL DE CAMPINAS
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ANA KAROLINE FERREIRA IGNÁCIO CÂMARA

REDUCTION OF FAT AND PHOSPHATE IN MEAT PRODUCTS EMULSIFIED THROUGH THE USE OF CHIA MUCILAGE (*SALVIA HISPANICA* L.)

REDUÇÃO DE GORDURA E FOSFATO EM PRODUTOS CÁRNEOS EMULSIONADOS ATRAVÉS DA UTILIZAÇÃO DA MUCILAGEM DE CHIA (*SALVIA HISPANICA* L.)

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Orientadora: Dra. Marise Aparecida Rodrigues Pollonio

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RESUMO

A elevada ingestão de gordura saturada é uma preocupação recorrente, considerada alto fator de risco para o desenvolvimento de doenças crônicas como as cardiovasculares, diabetes e hipertensão. Várias diretrizes alimentares recomendam limitar o consumo de produtos cárneos devido ao seu teor de gordura saturada, além da presença de outros constituintes considerados prejudiciais como sal e fosfatos. Estratégias tecnológicas de redução/substituição destes constituintes podem auxiliar na elaboração de produtos cárneos mais saudáveis. A mucilagem de chia é um ingrediente funcional com perspectivas promissoras, tanto para substituição da gordura como redução de fosfatos, devido à sua capacidade de retenção de água e formação de gel. Este trabalho teve como objetivo estudar a utilização da mucilagem de chia em suas diferentes formas de aplicação (em pó, na forma de géis e emulsões géis (EGs)) sobre as propriedades tecnológicas, sensoriais, nutricionais, microestruturais e fisiológicas em produtos cárneos emulsionados com redução de gorduras e fosfatos. Primeiramente, a mucilagem de chia (MC) foi caracterizada e suas propriedades reológicas avaliadas, comparando seus efeitos individuais com sistemas modelos cárneos reformulados. A MC apresentou-se como um gel fraco com comportamento reológico viscoelástico e propriedades mecânicas estáveis após estresse térmico. Sistemas modelos adicionados de mucilagem de chia gel (MCG) foram mais firmes e estáveis, menos elásticos e coesos. O potencial da MC para reduzir fosfatos também foi avaliado em mortadelas com redução de gorduras, assim como a melhor forma de adição (em pó ou gel). Melhores propriedades de textura e aceitação global foram obtidas em tratamentos com adição de até 2% de MCG. Atributos de cor sofreram alterações indesejáveis com adição da MC, com menores valores de L* e a*. Acima deste nível (4%), as amostras apresentaram estrutura menos coesa e organizada, com mobilidade da água mais restrita. EGs contendo MC como principal agente gelificante e azeite de oliva, combinadas com diversos compostos, foram avaliadas para reduzir o teor de gordura em mortadelas. Alginato de sódio, colágeno e proteína do soro de leite foram selecionados para elaboração das EGs levando-se em consideração as melhores propriedades tecnológicas. Os efeitos das EGs foram avaliados em mortadelas reduzindo os níveis de gordura e fosfatos em 100% e 50%, respectivamente. Melhor perfil de ácidos graxos, com redução de saturados e aumento nos teores de ω-9, alta estabilidade de emulsão e aceitação sensorial semelhante ao controle foi encontrada nos produtos com EGs. Por fim, as propriedades de saciedade e comportamento durante análise de

digestão *in vitro* da MC foram investigadas em mortadelas com MCG e EGs e redução de gordura. Sensações de apetite semelhantes foram percebidas pelos voluntários (P> 0,05) para todos tratamentos. Maior digestibilidade proteica (83,80%) foi encontrada para o controle, que diferiu (P <0,05) dos demais. Imagens de microscopia revelaram uma maior agregação das amostras com MC na fase gástrica, demonstrando interferência na bioacessibilidade das proteínas. A partir dos resultados obtidos, concluiu-se que a MC apresenta enorme potencial para ser explorada como substituto de gordura inovador e uma alternativa para redução de fosfatos, com propriedades nutricionais benéficas quando aplicada em produtos cárneos emulsionados.

Palavras-chave: mucilagem de chia; redução de gordura; mortadela; redução de fosfato; emulsão gel; saciedade.

ABSTRACT

High intake of saturated fat is a recurring concern, considered a high-risk factor for the development of chronic diseases such as cardiovascular, diabetes, and hypertension. Several dietary guidelines recommend limiting the consumption of meat products due to their saturated fat content, as well as the presence of other harmful constituents such as salt and phosphates. Technological strategies for reducing/replacing these constituents may assist in the development of healthier meat products. Chia mucilage presents itself as a functional ingredient with a promising perspective, both for replacing fat and reducing phosphates in meat products, due to its high-water retention capacity and gel formation. This study aimed to study the use of chia mucilage in its different forms of application, that is, in the form of powder or gels, and emulsion gels (EGs) on the technological, sensory, nutritional, microstructural and physiological properties in emulsified meat products with reduced fat and phosphates. First, chia mucilage (MC) was characterized, and its rheological properties were evaluated, comparing its individual effects with reformulated meat model systems. The MC presented as a weak gel with viscoelastic rheological behavior and with stable mechanical properties after thermal stress. Systems models added of chia gel mucilage (MCG) were firmer and stable, less elastic and cohesive. The potential of MC to reduce phosphates was also evaluated in Bologna sausage with fat reduction, as well as the best form of addition (powder or gel). Better texture properties and global acceptance were obtained in treatments with the addition of up to 2% of MCG. Color attributes have undesirable alterations with the addition of MC, with lower values of L* and a*. Above this level (4%) the samples presented a less cohesive and organized structure, with shorter relaxation times and more restricted water mobility. EGs containing MC as the main gelling agent and olive oil were also evaluated to reduce the fat content in Bologna sausage. We studied the elaboration of EGs with MC combined with several compounds. Sodium alginate, collagen, and whey protein were selected for the elaboration of the EGs, taking into account the best technological properties. The effects of the EGs were evaluated in Bologna sausage, reducing the levels of fat and phosphates in 100% and 50%, respectively. Better fatty acid profile with saturated fatty acids reduction and increased ω-9 contents, high emulsion stability, and similar sensory acceptance to control were found in the products with EGs. Finally, the satiety properties of MC were investigated in Bologna sausage with MCG and EGs and fat reduction, in addition to the behavior of products submitted to *in vitro* digestion.

Similar appetite sensations were perceived by the volunteers ($P > 0.05$) for all treatments. Higher protein digestibility (83.80%) was found for the control, which differed ($P < 0.05$) of the others. Microscopy images revealed a higher aggregation of the samples with MC in the gastric phase, demonstrating interference in the bioaccessibility of proteins. Based on the results obtained, it was concluded that MC has an enormous potential to be exploited as an innovative fat substitute and an alternative to reduce phosphates, with beneficial nutritional properties when applied to emulsified meat products.

Keywords: chia mucilage; fat reduction; Bologna sausage; phosphate reduction; emulsion gel; satiety.

SUMÁRIO

INTRODUÇÃO GERAL

A obesidade é considerada um alto fator de risco para o desenvolvimento de doenças crônicas como as cardiovasculares, diabetes tipo 2, certos tipos de câncer, hipertensão, dislipidemia, dentre outras (BHAT et al., 2019). As taxas de sobrepeso e obesidade aumentaram de forma considerável nos últimos 35 anos, configurando um cenário preocupante, à medida que mais de um terço da população mundial atualmente é classificada como obesa ou com sobrepeso. Este elevado índice resulta de uma interação complexa entre diversos fatores tais como mudanças na oferta e disponibilidade dos alimentos, hábitos alimentares, estilo de vida sedentário, fatores socioeconômicos, ambientais e genéticos (CHOOI et al., 2019).

Considerando esse contexto, a demanda por alimentos mais saudáveis por consumidores cada vez mais conscientes da relação entre dieta e saúde, aumentou de forma significativa. A indústria de alimentos tem desenvolvido novos produtos com apelos mais saudáveis que visam atender atendam a estas demandas. Carnes e produtos cárneos são frequentemente percebidos como não saudáveis, porém, muitas das conotações negativas associadas a estes produtos podem ser superadas pela redução/substituição dos constituintes considerados prejudiciais, como gorduras saturadas, sal e fosfatos (GRASSO et al., 2014). Produtos cárneos emulsionados, como mortadelas e salsichas, amplamente consumidos em todo o mundo, são fontes de proteínas, ácidos graxos mono e polinsaturados, vitaminas e minerais e possuem grande importância econômica para a indústria cárnea. No entanto, têm recebido sérias críticas ao seu consumo em função de seus tradicionais teores elevados de gorduras, entre 20-30%, constituindo uma excelente oportunidade para a reformulação (PINTON et al., 2019; JIMÉNEZ-COLMENERO, 2000).

Embora esta necessidade de reformulação crie diversas oportunidades, exemplificadas por muitas pesquisas bem-sucedidas na redução de gordura e aditivos, muitos desafios impedem uma aplicação mais abrangente do ponto de vista industrial e prático (GRASSO et al., 2014). A gordura de origem animal, particularmente a suína, em grande parte definida como toucinho, desempenha um papel essencial na elaboração dos produtos cárneos e, sua substituição ou redução é sempre desafiador do ponto de vista tecnológico. A gordura interage com os outros ingredientes da matriz cárnea e auxilia no desenvolvimento da textura, palatabilidade global, principalmente, sabor (TOBIN et al., 2012). Em produtos cárneos emulsionados, soma-se a estes efeitos a estabilidade dos produtos, com menores perdas na

cocção e menor susceptibilidade à oxidação lipídica devido a maior concentração de ácidos graxos saturados (JIMÉNEZ-COLMENERO, 2000; TOBIN et al., 2012).

Além dos desafios enumerados acima inerentes de uma reformulação lipídica em produtos cárneos, há outra tendência igualmente atual e desafiadora, que é a redução de aditivos nestes produtos, comumente chamada de "clean label", ou seja, rótulos mais limpos.

Estima-se que cerca de 50% da ingestão diária de fósforo (P) seja proveniente de aditivos alimentares, como os fosfatos, que são muito utilizados pela indústria cárnea (WINGER; URIBARRI; LLOYD, 2012). Os fosfatos são aditivos sintéticos que exercem funções primordiais em produtos cárneos como o aumento da capacidade de retenção de água, e, consequentemente, redução das perdas na cocção e aumento da suculência, além de ser um eficiente agente antioxidante (ABERLE et al., 2001). Desta maneira, a redução deste aditivo em produtos cárneos, apesar de desafiadora, é bastante necessária. A alta ingestão de fosfatos pode colocar em risco a saúde de um grupo específico de consumidores, tais como as pessoas com doenças renais crônicas (BENINI et al., 2011), ou também estar associada à aterosclerose coronariana na população em geral (RITZ et al., 2012).

Estratégias tecnológicas utilizadas para desenvolver produtos cárneos mais saudáveis são baseadas, mais comumente, na substituição da gordura por ingredientes não cárneos, como fibras alimentares ou hidrocolóides e proteínas de origem animal ou vegetal, que podem conferir características texturais desejadas e alcançar certos atributos funcionais ou influenciar a composição final do produto (OLMEDILLA-ALONSO; JIMÉNEZ-COLMENERO; SÁNCHEZ-MUNIZ, 2013). Tais compostos funcionais podem ter propriedades, como por exemplo, alta capacidade de retenção de água (fibras alimentares), úteis também para compensar a ausência ou redução de aditivos, como os fosfatos (SLAVIN, 2005; CHOE et al., 2018).

Nesse contexto, a semente de chia (*Salvia hispanica* L.) oferece considerável potencial para o desenvolvimento de alimentos mais saudáveis devido às suas propriedades nutricionais, como teor de óleo (entre 30-33%) e composição de ácidos graxos (aproximadamente 80% do total são ácidos graxos insaturados), carboidratos (26-41%), fibras alimentares (18-30%), proteínas (15-25%) e minerais (4-5%) (SEGURA-CAMPOS et al. 2014). A chia tem sido utilizada de forma incipiente como ingrediente em produtos cárneos sob a forma de farinha, sementes inteiras ou a farinha incorporada em emulsões géis (BARROS et al., 2018; DING et al., 2018; PINTADO et al., 2016). No entanto, não foram encontradas referências relevantes na literatura ao uso da mucilagem de chia em produtos cárneos.

A mucilagem de chia (MC) é um polissacarídeo de alto peso molecular extraído das sementes. Quando em contato com a água, a mucilagem é formada e lixiviada para fora do pericarpo e a viscosidade da solução aumenta bastante. Este polímero é composto por β-D-xylose, α-D-glicose e 4-O-mehtyl-α-D-ácido glicurônico na proporção de 2:1:1, respectivamente (LIN; DANIEL; WHISTLER, 1994; MUÑOZ et al., 2012). Esse componente pode se tornar um ingrediente muito promissor para reformulações em produtos cárneos mais saudáveis devido às suas propriedades funcionais e fisiológicas. Entre suas propriedades funcionais, destacam-se sua alta capacidade de retenção de água, capacidade emulsificante, alta solubilidade, agente estabilizante e espessante (CAPITANI; NOLASCO; TOMÁS, 2016; GOH et al., 2016; VÁZQUEZ-OVANDO et al., 2009). Em relação às suas propriedades fisiológicas, a mucilagem de chia, sendo uma fibra alimentar solúvel, forma géis de alta viscosidade que produzem distensão gástrica, esvaziamento mais lento do estômago e sensação de saciedade (HOAD et al., 2004).

Apesar da potencialidade, os estudos com a mucilagem de chia são recentes e, ainda, há um vasto campo de pesquisa a ser explorado com este ingrediente em produtos cárneos, haja vista as inúmeras possibilidades de aplicação, combinações com outros ingredientes e avaliação dos seus efeitos fisiológicos como saciedade e digestibilidade. Os desafios no desenvolvimento de produtos cárneos mais saudáveis sempre estiveram presentes e, muitos avanços estão sendo alcançados, porém, a forma de avaliar estes compostos e as questões levantadas pela sociedade e pela comunidade científica estão mudando. Além das respostas tecnológicas, sensoriais e de segurança, é importante buscar mais conhecimentos de como novos ingredientes interagem com a matriz do alimento, em nosso caso dos produtos cárneos e como uma reformulação pode afetar a biodisponibilidade de nutrientes e as percepções de saciedade e apetite. A proposta e o desenvolvimento deste projeto possibilitaram a geração de conhecimentos inéditos sobre o comportamento da mucilagem de chia em produtos cárneos com redução de gordura e fosfatos, tanto do ponto de vista tecnológico quanto fisiológico.

O presente trabalho está organizado na forma de capítulos, de acordo com as etapas desenvolvidos no projeto, assim apresentados:

O capítulo 1 (artigo 1) consiste em um artigo de revisão que aborda os desafios e critérios para estudos com substitutos de gordura em produtos cárneos tais como as mucilagens e outros compostos funcionais de plantas bem como os efeitos sobre as propriedades tecnológicas e mecanismos relacionados à regulação do apetite e digestibilidade associados ao uso desses ingredientes inovadores.

O capítulo 2 (artigo 2) tem por objetivo caracterizar a mucilagem de chia como substituto de gordura em sistemas modelos cárneos emulsionados, bem como avaliar o comportamento reológico de dispersões de mucilagem.

O capítulo 3 (artigo 3) investigou o efeito da adição da mucilagem de chia em produtos cárneos emulsionados com baixo teor de gordura como uma estratégia tecnológica "clean label" com redução ou ausência de fosfatos, com determinação das possíveis diferenças e impactos tecnológicos e sensoriais em sua forma de adição (em pó ou gel).

O capítulo 4 (artigo 4) avaliou as possiblidades de utilização da mucilagem de chia como ingrediente em emulsões géis em combinações com outros agentes gelificantes através da avaliação de diversos parâmetros tecnológicos e reológicos importantes na aplicação em produtos cárneos.

O capítulo 5 (artigo 5) investigou os efeitos da substituição total da gordura e a redução de 50% nos níveis de fosfatos em mortadelas elaboradas com emulsões géis à base de mucilagem de chia em combinação com agentes gelificantes selecionados no capítulo 4 (colágeno, alginato e proteína do soro de leite) sobre as propriedades tecnológicas, sensoriais, microestruturais e de mobilidade da água.

O capítulo 6 (artigo 6), por fim, consistiu em avaliar mortadelas reformuladas com mucilagem de chia e emulsão gel à base de mucilagem de chia (formulações selecionadas nas etapas anteriores) em relação às percepções de saciedade de voluntários saudáveis bem como investigou as mudanças ocorridas nos produtos durante uma simulação de digestão *in vitro.*

Espera-se com esse trabalho, contribuir com bases científicas para o desenvolvimento de produtos cárneos mais saudáveis através do uso de ingredientes inovadores e estratégias de reformulação aplicáveis pela indústria de processamento com reais benefícios para os consumidores e, com isso, promover avanços nos setores socioeconômicos e de Saúde Pública.

OBJETIVOS

Objetivo geral

Este trabalho teve como objetivo geral estudar a utilização da mucilagem de chia em suas diferentes formas de utilização, ou seja, em pó, na forma de géis e emulsões géis sobre as propriedades tecnológicas, sensoriais, nutricionais, microestruturais e fisiológicas em produtos cárneos emulsionados com redução de gorduras e fosfatos.

Objetivos específicos

- Caracterizar a mucilagem de chia e estudar as suas propriedades funcionais e reológicas visando sua aplicação como substituto de gordura animal e fosfatos.
- Investigar o efeito da adição de mucilagem de chia em mortadelas como substitutos de gordura e fosfatos sobre as propriedades tecnológicas e sensoriais.
- Avaliar o efeito da adição de mucilagem de chia em emulsões géis à base de azeite de oliva e diferentes hidrocolóides a serem utilizadas como substitutos de toucinho em mortadelas.
- Compreender as interações que ocorrem em um sistema cárneo emulsionado com redução de gordura animal adicionado de mucilagem de chia durante um processo de digestão *in vitro*.
- Avaliar o potencial de saciedade fornecida pela mucilagem de chia como substituto de gordura em produto cárneo emulsionado.

CAPÍTULO 1

REDUCING SATURATED FAT IN EMULSIFIED MEAT PRODUCTS BY FUNCTIONAL INGREDIENTS: THE CHIA (*SALVIA HISPANICA* L.) MUCILAGE AS A HEALTHY STRATEGY

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Reducing saturated fat in emulsified meat products by functional ingredients: the chia (Salvia *hispanica* L.) mucilage as a healthy strategy

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Abstract

The prevalence of obesity, excess body weight and obesity-related diseases is increasing worldwide. The reduction or removal of fat in meat products is desirable from a health perspective since high consumption is commonly associated with many chronic illnesses. There has been a considerable information of scientific data concerning to adding specific components with potential health implications for fat reduction for development of functional meat products. However, there are also very few studies involving these meat products to verify whether those modifications are of relevance to human health. In this review, the behavior of chia mucilage, as well as of their derivatives, besides other mucilages and seeds, as new fat substitutes in meat products are discussed, and the physiological implications of such behavior are highlighted. Thus, the paper addresses critical issues such as the challenges in developing healthier meat products with a focus on the central mechanisms for regulating the appetite and bioavailability of new fat substitutes, as well as crucial issues addressing the technological aspects of the application, challenges and consumer perception about reformulations of meat products.

Keywords: meat products; functional ingredients, chia mucilage, satiety, digestibility, healthy, new fat substitutes

1. Introduction

The prevalence of obesity, excess body weight and obesity-related diseases is increasing worldwide. Obesity is considered a complex multifactorial disease. The global prevalence of overweight and obesity has doubled since 1980 to an extent that nearly a third of the world's population is now classified as overweight or obese (Chooi, Ding, and Magkos, 2019). The simultaneous rise in excess body weight in almost all countries is thought to be driven largely by changes in the global food system, which promotes energy-dense, nutrient-poor foods, alongside an increased sedentary lifestyle (Afshin et al., 2019). In this context, many dietary guidelines recommend limiting the consumption of processed foods that are rich in fat, and particularly, saturated fatty acids. In this scenery, meat products have been described as non-healthy foods due to these characteristics (USDA, 2015; Aranceta and Pérez-Rodrigo, 2012).

According to Olmedilla-Alonso, Jiménez-Colmenero, and Sánchez-Muniz (2013), meat and meat products are excellent food systems for reformulation, due to the quantity and frequency of consumption, the contribution to the dietary intake of different nutrients, the convenience and sensory properties and, mainly, the opportunity to change the bad nutritional image by incorporation of natural ingredients.

Thus, during the last two decades, there has been a considerable number of scientific data concerning to optimize the presence of specific components with potential health implications, and for reduction fat for development functional meat products (De Smet and Vossen, 2016; Olmedilla-Alonso et al., 2013). Many fat reducing strategies have been investigated in meat products by adding different substitutes e.g. inulin (Keenan et al., 2014) and konjac gel (Jiménez-Colmenero et al., 2012), oat fiber and carrageenan (Hugues, Cofrades, and Troy, 1997), rye bran (Yilmaz, 2004), canola oil and pre-emulsified canola oil (Youssef and Barbut, 2011), cellulose, carboxymethyl cellulose, chitosan, and pectin (Han and Bertram, 2017), among others. In these studies, several technological and sensorial advances were obtained, such as improvement of stability, reduction of cooking losses, and obtaining products with sensorial attributes similar to traditional formulations (Yilmaz, 2004; Schmiele et al., 2015, Choi et al., 2009). Also, there were significant advances for the elucidation and an understanding of the interactions between the components at a microstructural level (Han and Bertram, 2017).

Besides, more recently, new fat substitutes have emerged in scientific researches as an attempt to mimic the effects that animal fat provides to meat products as palatability, taste, juiciness, and stability, without however increasing the levels of saturated fatty acids, such as emulsion gels, hydrogels, and organogels (Paglarini et al., 2019; Alejandre et al., 2019; Heck et al., 2019). Just as there has been a greater valorization of plant mucilage (seed coat or cladodes extracted) as potential structuring and texturizing agents for food or nutraceutical applications has gained recently much attention (Soukoulis, Gaiani, and Hoffmann, 2018)). Even so, the application of these mucilages as fat substitutes in meat products is incipient and few studies are reported (Saengphol and Pirak, 2018). Besides, there are very few studies involving these meat products to verify whether those modifications are of relevance to human health (De Smet and Vossen, 2016; Olmedilla-Alonso et al., 2013).

The mucilage extracted from chia seed, considered a soluble fiber and, by its chemical composition a hydrocolloid, can become an ingredient with favorable perspectives in the general food industry and, specifically, in the meat industry as a potential substitute for fat to be investigated. Chia mucilage has functional properties such as high-water retention

capacity, thickening properties, emulsifying, and stabilizing capacity (Wu et al., 2010; Vázquez-Ovando et al., 2009). Furthermore, this mucilage is a natural heteropolysaccharide considered safe for use in food, with biocompatibility and biodegradability, offering opportunities for the industry to use in its products "cleaner labels" (Elboutachfaiti et al., 2017). The soluble fibers have the functional property of forming viscous mixtures in the gastrointestinal tract, thus being directly related to the promotion of satiety. Also, they affect the absorption of glucose and lipids in the small intestine, which are easily fermented by bacteria in the colon (Slavin, 2005).

Studies and reviews on nutritional, rheological and functional aspects of the chia mucilage and chia and coproducts can be found in the scientific literature, but the application of these functional compounds in meat products is still scarce. Physiological effects such as satiety and digestibility of new fat substitutes applied in meat products require a more detailed report. There is a lack of systematic review of the impacts of new fat substitutes, such as mucilages, organogels and emulsion gel in meat products with central mechanisms for appetite regulation, and studies in this area are still very beginning. In this review, the behavior of chia mucilage, as fat substitutes in meat products regarding its technological, functional and sensory effects is described correlating its potential use as natural ingredient in meat products as well its challenges to replace animal fat. Furthermore, the physiological implications are highlighted by approach related to satiety and digestibility, relevant issues concerning to new fat substitutes used in strategic reformulations aiming healthy claims. We hope to address critical topics to evaluate in a reliable view the possibilities to develop new meat products from a promising functional ingredient derived from chia seeds.

2. Technological challenges and strategies to reduce saturated fat in meat products

Animal fat, particularly pork back fat, plays a key role in the production of meat products, and their replacement or reduction is always technically challenging. The fat interacts with the other ingredients of the meat matrix and assists in the development of texture, palatability, besides contributing to the flavor (Tobin et al., 2012). In emulsified meat products, the stability of the products is added to these effects, with lower cooking losses and lower susceptibility to lipid oxidation due to a higher concentration of saturated fatty acids (Jiménez-Colmenero, 2000; Tobin et al., 2012).

Besides to the challenges listed above inherent in a lipid reformulation in meat products, there is another trend quite current and challenging in the same way, which is the reduction of additives in these products, commonly called "clean label." Meat emulsions are formed from the homogenization of meats, water, a source of fat, usually the pork back fat, in addition to different ingredients allowed by the legislation, as several additives, being the phosphates one of the most important from the point of view functional. The myofibrillar proteins during the comminution process are solubilized by sodium chloride and also by phosphates, which then form a protein film around the fat globules. In this process, a source of fat with a high melting point, as well as the performance of additives is highly desirable to ensure the stability of the protein network and the final product (Aberle et al., 2001). For this reason, the substitution of pork back fat is quite challenging in meat products, mainly when it aims to meet the trend of clean label, that is, healthier products, with fewer fats and additives.

Based on this technological reason, pork back fat is the main fat source of meat products with about 27% of palmitic acid (C16:0), 16% stearic acid (C18:0), 45% oleic acid (C18:1), and 7% linoleic acid (C18:2) (Valsta, Tapanainen, and Männistö, 2005). A good quality fat has to be white and firm, stable to lipid oxidation and presents a characteristically flavor to ensure the final quality of the meat products. Changes in the fatty acids composition of pig adipose fats may modify the consistency of meat products and their stability towards oxidation (Hugo and Roodt, 2007). The firmness of pork back fat is positively correlated with stearic acid C18:0 (Liu, Lampi, and Ertbjerg, 2018) and inversely correlated with C18:2. Besides that, a higher concentration of polyunsaturated fatty acid in pork back fat will result in a product more susceptible to lipid oxidation (Hugo and Roodt, 2007).

The fat content of meat products can range from 5-50g /100g (Jiménez Colmenero, 2000). Figure 1 shows the fatty acid profile of some meat product (TACO, 2011; USDA, 2018). It can be observed that the group comprising the restructured products (chicken nugget, beef burger) present a fat content between 12 and 21g /100g, while raw and cooked cured products (fresh sausage, ham) have a fat content lower than 10g /100g. The firm raw sausages (salami) have values close to 35 g/100g and cooked sausages (Bologna, Frankfurters, liver sausage) have around 20–30 g fat/100 g product.

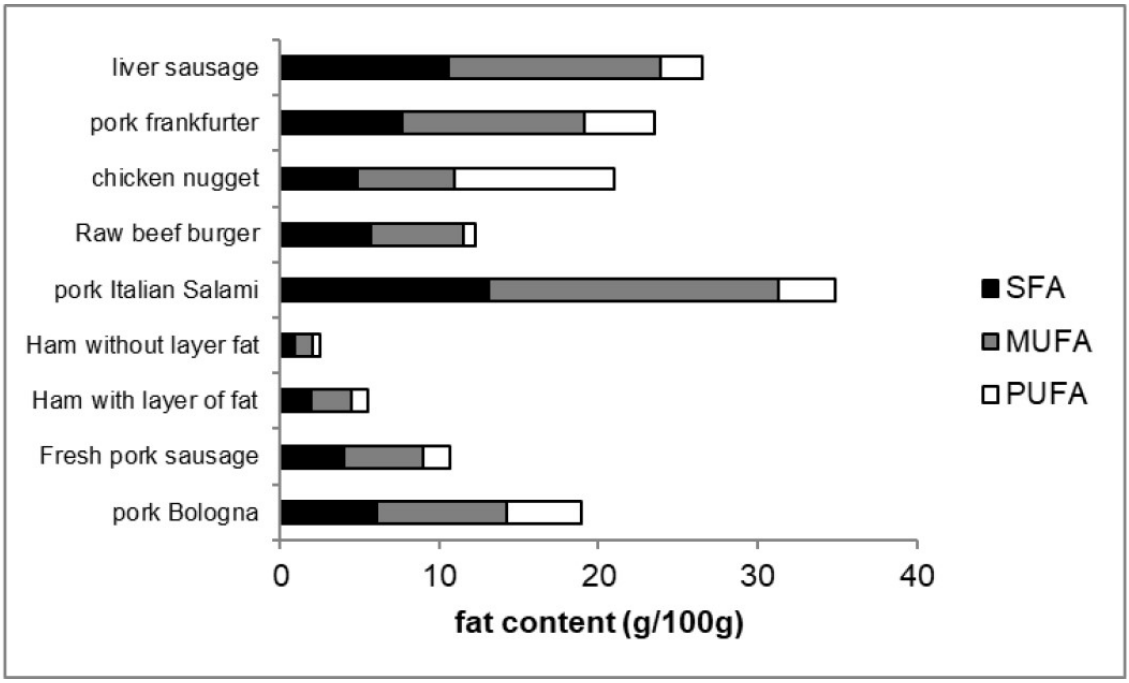

Figure 1. Fatty acid profile of different meat products. Data from USDA (2018) and TACO (2011)

Animal fats are frequently mentioned when it comes to reducing the proportion of fat in the diet, however, fat play an essential role in meat products (Tobin, et al., 2012). Despite being responsible for many desirable technological qualities in fats, a high percentage of saturated fatty acids (SFA) and cholesterol content in meat fat is linked to negative health effects (Schmid, 2010). A high proportion of the ω-6 / ω-3 ratio is currently found in the Western diet (Simopoulos, 2010), and pork back fat widely present in meat products contribute to this fatty acid profile ratio in the diet. The balance between these fatty acids is essential for homeostasis and healthy development of the body. A rate equal to less than 5:1 has been recommended (Bernardi et al., 2016). Therefore, it is desired to reduce SFA levels and increase ω-3 levels in processed meat products in order to obtain products that are healthier by consumers without affecting the products' sensory characteristics (Ospina-E, et al., 2015).

However, to substitute pork back fat to provide a consistent fat reduction can result in some technological problems. Meat products such as Bolognas and Frankfurters are emulsion-type sausages and on the average contain 25% of fat presents in emulsified and suspended form (Hugo and Roodt, 2007). In these products, fat has an significant role in affecting the quality, such as cooking loss, texture, color, flavor, sensory, and shelf life, and then, it cannot be easily reduced and/or modified by using less fat or replacing animal fat with another type of fat (Jiménez Colmenero, 2000; Tobin et al., 2012). For example, an excessive

fat reduction produces hard (reduced juiciness) or rubbery products because of weight loss (Wang et al., 2016), present reduced cook yields, soft and mushy interiors, rubbery skin formation, excessive purge in vacuum packages, shortened shelf-life and changes in mouthfeel of the product after conventional or microwave reheating (Keeton, 1994).

Several strategies have been extensively studied to reduce and / or replace saturated fat, particularly pork back fat, in meat products. The easier strategy to reduce saturated fat in this context is using lean cuts and simple fat reduction adding water to compensate the pork back fat removed, being mostly of the times, made unfeasible by the considerable increase in cost (Olmedilla-Alonso et al., 2013). Many studies have reported adding extender ingredients (fibers, hydrocolloids, starches) directly in formulations aiming to improve the water holding capacity and gel and emulsion properties in reduced fat meat product (Schmiele et al., 2015; Choe et al., 2013). But the strategy most investigate suggest the incorporation of vegetable or marine oils, a technique that results in loss of texture and increase of lipid oxidation (Selani et al., 2016). However, in summary, such research lines have been described many defects associated with loss of taste, aroma, juiciness, texture, heat transfer, processing operations depending on the partial or total removal of saturated fat in the formulation.

Although the incorporation of vegetable oils as a fat replacer has considerable nutritional advantages, the presence of less saturated fats or oils with high levels of monounsaturated fatty acids (oleic acid) is associated with changes in the physicochemical properties of the fat (melting point, color, flavor) (Jiménez-Colmenero, 2000). It also can accelerate the lipid oxidation reaction, especially, in the presence of oxygen during mechanical processing such as grinding or chopping, cooking treatments, and addition of salt during the processing procedures (Álvarez et al., 2011).

In this scenery, the development of emulsion gels appears as a strategy to overcome instability of fat substitutes composed predominantly of vegetable oils and water in systems that require the texture properties conferred by solid fats such as meat products emulsified with fat reduction (Jiménez-Colmenero et al., 2015).

An emulsified lipid gel can be defined as an emulsion containing a gel-like network structure and mechanical properties similar to that of a solid (Dickinson, 2012; 2013), basically formed in two steps. In the first, the formation of an emulsion containing oil + water added from a protein source as surfactant occurs. This mixture is submitted to high homogenization pressure or high mixing speed in mixers, cutters or similar equipment. To obtain a more stable lipid gel, other ingredients may be added to the newly formed emulsion such as an aqueous solution / dispersion of protein, surfactant or polysaccharide. In the second stage, gel formation

will occur from the emulsion obtained in the 1st stage by the aggregation of the droplets of the emulsion or by the gelation of the continuous phase, when using an appropriate heat treatment. The transformation of the liquid state to the soft solid state of protein-based emulsions results from processing steps such as heating, acidification or enzymatic treatment (Dickinson, 2013).

In meat products, the use of emulsified lipid gels is a very promising technique, since the objective in the meat matrix is to reduce saturated fat and / or improve the lipid profile and its application. If an appropriate method is conducted, it may allow the use of stabilized vegetable oils in a stable structure to act as fat substitute without technological problems.

Some studies have searched an adequate selection of gelling / emulsifying agents and ingredients with functional properties that remain stable after the processing conditions and do not alter the identity and quality pattern of the fat-emulsified meat product. Among the structuring agents most studied in the development of emulsion gels are proteins, different hydrocolloids and functional ingredients, especially sodium caseinate, serum proteins, soy proteins, gelatin, lecithin, carrageenan, inulin, among others (Jiménez-Colmenero et al., 2010; Paglarini et al., 2019; Pintado et al., 2015). For this purpose, studies with chia mucilage as structuring agent are quite limited, but this could be an excellent strategy to be investigated in order to bring more consistent claims for healthy consumption.

3. Fat reduction in meat products and consumer´s perception

Meat and meat products generally have high nutritional value, considering their protein, fatty acids, minerals and vitamins content (Biesalski, 2005; Decker and Park, 2010; Li et al., 2005). However, they are often associated to increased risk of some diseases due to their high amount of fat, salt and additives. Hypertension, obesity, cardiovascular disease and cancer have been associated to high levels of meat products consumption (Corpet, 2011; McAfee et al., 2010; Oostindjer et al., 2014; Rohrmann et al., 2013).

Reducing fat, in particular, saturated fat appears as one of the most important reformulations to be conducted to achieve healthier claims. Public health campaigns in many countries have alerted population to reduce fat, influencing both in research and industry to purpose alternatives to develop reformulated products (de Barcellos et al., 2011). In this sense, some strategies have been explored with health-promoting components, which demands great technological effort due to the excellent technological properties from the animal fat. The functional properties regarding emulsion and gel formation, the stability resulted from a high

melting point and, sensory properties, are the main challenges to be solved when saturated fat is replaced in meat products. Using vegetable oils, fibers, hydrocolloids, emulsion gel, and organogels are some of strategies that have been studied in different meat product categories (Câmara and Pollonio, 2015; Mora-Gallego et al., 2016; Rodríguez-Carpena et al., 2012; Schmiele et al., 2015). To produce a meat product with equal identity patterns means is the most concern because these characteristics will result in acceptation by consumers, an essential condition to achieve success next to consumers.

It's not easy to determine consumer's acceptance of a healthier meat product. It depends on demographic characteristics, culture, sensory quality, and "traditional" characteristics (Costell et al., 2010; Luckow et al., 2005; Shepherd and Sparks, 1994). In general, the sensory acceptance is related to how it responds to consumer needs and degree of satisfaction when eating (Schnettler et al., 2019; Heldman, 2004).

In one study developed in questionnaire forms, Fellendorf et al., (2018) concluded that consumers are aware of the risks that a diet rich in fat can bring, and also, fat reduced foods are well accepted. In Chile, participants were asked if they were willing to pay a higher price for a frankfurter with a different claim, such as low sodium, low fat or added by fiber (Schnettler et al., 2019), however, the results showed that they were not willing to pay more. Shan et al. (2017) also concluded that price and base meat influence more in purchase intention than reformulation with a healthy claim.

Considering price and sensory quality, consumers are already searching for alternatives for healthier meat products (Schnettler et al., 2019), that combine sensory acceptance with minor modifications in texture, aroma and color (Jiménez-Colmenero, 2000). Although these parameters can be affected, overall acceptability may be maintained as it is shown in some studies.

In semi-fermented dry sausages added by wheat fiber, Ores et al. (2018) found that the addition of wheat fiber did not influenced the overall acceptability and buying intention of these products, however, the addition of 1.5 or 3% of wheat fiber, combined with buffalo or bovine meat influenced in colour and flavour. Arnarson et al. (2011) investigated sensory acceptance in meat products developed with fat and/or salt reduction, to people with restricted diet (obese individuals). Consumers, despite the differences in aroma, texture, and appearance, the study indicated that such fat-reduced meat products were well accepted.

Alves et al. (2016) produced low fat bologna sausages with pork skin and green banana flour and they observed that depending on the levels of substitution, good sensory

quality was observed. Up to 60% of substitution of pork fat, sensory acceptance was maintained to consumers, regarding overall acceptability, colour, aroma and texture.

Considering fat substitution in breakfast sausages by the addition of different levels of pea extract, a sensory analysis was conducted in different age groups, in a studied performed by Conroy et al., (2018) in Ireland. The fat reduction (15% pork back fat) was most preferred in 41–64 yr. olds and elderly people (65+ yr.old) preferred 30% fat sausages. This fact indicates the necessity to understand different age groups and meat products reformulation, but also indicates the possibility to reduce fat without compromising sensory acceptability.

Tobin et al. (2013) studied the impact of fat reduction in breakfast sausages, showing that sausages were darker, tougher and less juicy compared to sausages with high fat content. However, hedonic scale did not find any preferred, indicating that fat reduction is possible if texture was enhanced. One way to overcome this texture problem is by adding fat replacers that improve both texture and water retention, such as carrageenan and locust beam gum (García-García and Totosaus, 2008).

Depending of kind of substitution is performed, it can impact negatively on texture. As Saldaña et al. (2018) studied the sensory characteristics of low fat mortadellas using descriptive analysis and check-all-that-apply (CATA) questions and relating it to overall liking. Results showed that fat reduction did impacted in overall liking, and the gelatinous texture was identified as a negative attribute. Other studies also showed negative impact of reformulation over sensory acceptability (Shan et al., 2017; Żakowska-Biemans et al., 2016). Therefore, the studies show that there has been an evolution in the development of healthier meat products; however, challenges still need to be overcome to better acceptance consumers.

4. Functional plant-based compounds as fat substitute

Animal fat is an essential component in meat and meat products responsible for such quality characteristics as juiciness, mouthfeel, texture, flavor, aroma, and cooking yield (Choi et al. 2013). Furthermore, fat is mostly responsible for satiety, energy value, and is a vehicle for fat-soluble vitamins (Vural, Javidipour, and Ozbas, 2004). On the other hand, the relationship between saturated fatty acids and cholesterol with increased risk of developing cardiovascular diseases motivates their replacement by healthier ingredients (Mozaffarian, Appel, and Van Horn, 2011), like plant-based compounds.

Whole plant-based compounds are sources of dietary fibers, proteins, unsaturated fatty acids, vitamins, minerals and antioxidant compounds, like carotenoids and flavonoids that have been associated with reduced risk for CVD (Hu, 2003; Hygreeva, Pandey, and Radhakrishna, 2014). Beyond the nutritional aspects, plant components, as proteins, carbohydrates, and dietary fibers may have technological properties such as water-binding, thickness, stabilizer and emulsifier, which in part resembles the properties conferred by fat in meat products. Table 1 summarizes several studies reporting the fat replacement by whole plant-based compounds, such as flours and pastes of the seeds, nuts and beans and isolated plant-based compounds like dietary fibers, gums, and mucilages.

Nuts are a good source of unsaturated fatty acids, mostly monounsaturated and polyunsaturated fat, proteins that usually have a high content of the amino acid L-arginine, dietary fibers, vitamins as folic acid, niacin, vitamins E and B6, minerals as copper, magnesium and potassium and bioactive substances as phenols, carotenoids, flavonoids, and phytosterols (Blomhoff et al., 2006; Florowski et al., 2019; Ros, 2015). The Food and Drug Administration (FDA) suggests that eating 43 g/day of most nuts may reduce the risk of heart disease (FDA, 2003). According to Hu (2003) and Ros (2015), both the unsaturated fatty acids and the high content of arginine present in most nuts may protect against heart diseases; arginine is the precursor of nitric oxide, which is a potent vasodilator that can inhibit platelet adhesion and aggregation.

These nutritional properties make nuts an option to fat replacer in healthier meat products. Ayo et al. (2008) reported that 25% of walnut flour raised PUFAs, minerals, and dietary fibers contents of frankfurters. The cooking loss did not affect with walnut flour addition, but hardness and chewiness were influenced. Walnut conferred lower sensory scores related to texture and flavor, but acceptance did not differ for all frankfurters. (Saygi, Ercoşkun, and Şahin, 2018) reported that hazelnut paste (3-9%) improved the lipid profile of fermented sausages, but TBARS values raised with hazelnut added. However, the sensory properties of fermented sausages did not adversely affect by hazelnut addition. Florowski et al. (2019) analyzed various nuts on quality of restructured beef steaks; they showed that peanut, walnut, pecan nut and pistachios lowered cooking loss and shear force of steaks, and improved significantly nutritional value, mostly because of PUFAs and MUFAs raised. Overall acceptance did not differ between steaks added with the nuts previously reported and control sample, although the perception of nuts' flavor was remarkable.

Table 1. Meat products prepared by using plant-based compounds and main changes in these product

Plant-based compounds	Meat product	Changes in product	References
Pecan nut paste (6%)	Frankfurters	Pecan nut did not cause differences in proximal composition, lipid oxidation after 7 days of storage or lipid profile of the frankfurters.	Orozco et al. (2019)
Hazelnut paste (3-9%)	Fermented sausages	Hazelnut paste improved the lipid profile of sausages, but increased the lipid oxidation; sensory properties did not affect by replacing fat by hazelnut paste.	Saygi, et al. (2018)
Walnut flour (25%)	Frankfurters	Walnut flour increased PUFAs, minerals and dietary fibers of frankfurters; no difference in the cooking loss was achieved, but walnut flour increased hardness and chewiness values.	Ayo et al. (2008)
Poppy seed paste (5-20%)	Meat burgers	Poppy seed conferred better cooking yield, fat, and water retention at 20% level added, improved the lipid profile and mineral contents of meat burgers, and promoted better flavor and overall acceptance than the control.	Gök et al. (2011)
White, red and black quinoa pastes (5-7.5%)	Pâté	Quinoa pastes raised emulsion stability, moisture contents, hardness, and gumminess values; Pâté with 5% of the red quinoa was the most sensory acceptable sample.	Pellegrini et al. (2019)
Flaxseed flour (3-15%)	Beef patties	Flaxseed flour improved the lipid profile of the beef patties with minimal composition and sensory changes with 3% and 6% of the flaxseed flour added.	Elif Bilek and Turhan (2009)
Chitosan 4% and gold flaxseed flour (7.5%)	Hamburgers	Chitosan decreased yield, moisture retention, and sensory acceptance, but increased fat retention and texture parameters of the hamburgers. Flaxseed flour raised cooking yield, fat retention, and texture parameters and provided good sensory acceptance to hamburgers.	Hautrive et al. (2019)
Chia byproduct (8-12%)	Hamburgers	Chia byproduct changed the lipid profile, mainly raising PUFA content of the hamburgers, but lipid oxidation also raised with chia level.	Souza et al. (2015)
Adzuki bean flour (3.55-14.2%)	Beef meatballs	Adzuki bean flour raised cooking yield, hardness, and chewiness and decreased the calorie value and sensory acceptance of the meatballs.	Aslinah et al. (2018)

Table 1. Continuation

Plant-based compounds	Meat product	Changes in product	References
Chickpea, pea or wheat flour (2.5-5.0%)	Bologna sausages	Chickpea, pea and wheat flours at 5% level increased cooking yield and decreased purge loss; chickpea and pea flours improved the texture, but only chickpea was associated with desirable sensory texture properties.	Thushan Sanjeewa et al. (2010)
Pea flour, pea starch or pea fiber (4%)	Bologna sausages	Pea derivatives reduced cooking and purge loss; pea starch and fiber increased the texture profile of low-fat sausages comparable to the control sample; Sensory acceptance did not affect by pea starch and pea fiber addition.	Pietrasik and Janz (2010)
Blend of soy flour, Split-pea flour and wheat starch (15%)	Hamburger	Soy flour combined with wheat starch increased the cooking yield o the hamburgers; split-pea flour decreased shrinkage and improving texture profile.	Tabarestani and Tehrani (2014)
Hydrated wheat fiber (1.6-6.3%)	Beef burgers	The cooking loss was comparable to control for all formulations added wheat fiber; diameter reduction and texture parameters decreased with wheat fiber addition; wheat fiber promoted good sensory acceptable to hamburgers and satiety feelings was similar to the control.	Carvalho et al. (2019)
Apple fiber (3-4.5%)	Chicken patties	Apple fiber increased the cooking yield and decreased shortening and energy value without any change in shear force and sensory acceptance of chicken patties.	Guedes-Oliveira et al. (2016)
Pineapple fiber (1%) and water	Sausage	Pineapple fiber could lower the cooking loss and bind additional water in low-fat sausage formulations.	Henning et al. (2016)
Sugarcane dietary fiber (SDF) 1-3%	Meat batter	SDF improved water and fat binding properties and texture parameters; SDF added at 2% level had comparable overall acceptance to the control sample.	Zhuang et al. (2016)
Tragacanth gum (two varieties) (0.25-1%) and water	Sausage	Tragacanth gum lowered the cooking loss, extractable fat and water o the sausages; protein oxidation (express at carbonyl content) lowered over storage time with 1% of tragacanth gum; hardness decreased with gum raised; comparable sensory acceptance was achieved to sausages with tragacanth gum and high-fat control.	Abbasi et al. (2019)

Table 1. Continuation

Plant-based compounds	Meat product	Changes in product	References
Hoary basil seed mucilage gel (4.5-22.5%)	Chicken meat model	The replacement of pork back fat for HBM decreased the water holding capacity and hardness; HBM was able to substitute 80% of pork back fat without any change of sensory perception.	Saengphol and Pirak (2018)
Basil seed gum (BSG) (0.5%)	Myofibrillar protein gel	BSG raised cooking yield without changed the hardness, gel strength, color, and pH values.	Lee and Chin (2017)
Quince seed gum (QSG) (1.2-4.8%)	Hamburgers	QSG decreased cooking loss, TBARS value and raised firmness of the low-fat hamburgers.	Yousefi et al. (2018)

Beans and seeds are also an excellent source of nutrients, mainly proteins, dietary fibers, and phytochemicals. Chia (Fernández-López et al. 2019; Pintado et al. 2016, 2018; Souza et al. 2015), flax (Elif Bilek and Turhan, 2009; Hautrive et al., 2019), quinoa (Pellegrini et al., 2018), and poppy (Gök et al., 2011) are examples of seeds that may use as fat replacer in meat products. Chia seed and flaxseed are one of the highest whole food sources of dietary fibers, alpha-linolenic fatty acid, proteins with very similar amino acids contents, and micronutrients (Nitrayová et al., 2014).

Pintado et al. (2016) showed that chia flour incorporated at 10% level increasing dietary fiber, minerals, and linolenic acid contents of frankfurters and also decreased the cooking and purge losses. Hautrive et al. (2019) reported that flaxseed flour (7.5%) raised cooking yield, fat retention, and texture parameters and provided good sensory acceptance to hamburgers. According to the authors earlier mentioned, flaxseed and chia seed is a good source of soluble dietary fibers that have an excellent potential to act as hydrocolloid and raised the water holding ability in food systems.

Pellegrini et al. (2019) evaluated the nutritional and technological properties of some quinoa flours varieties; the protein content ranged from 11.62 to 13.66%, while the fat content was comprised between 4.87-6.48%, quinoa flours also presented some phenolic compounds. Despite their technological properties, quinoa flours showed lower water and oil holding capacities, but a high swelling capacity. The addition of quinoa paste (5-10%) in meat pâté as fat replacer raised emulsion stability, moisture contents, hardness and gumminess values, and protection against lipid oxidation.

These results were credited to water holding ability of quinoa after cooking and its antioxidant activity attributed to its bioactive compounds (Pellegrini et al., 2018). Gök et al. (2011) incorporated poppy seed paste (5-20%) to replace the fat in meat burgers. According to the authors, poppy seed paste improved lipid profile and mineral contents of the meat burgers. Poppy seed at 20% level raised cooking yield, fat and water retention of the burgers and conferred better sensory scores levels than the control sample.

Beans (as chickpea, pea, soy, and adzuki) and grains (as wheat, oat, and rice) likewise have good nutritional and technological properties to perform as fat replacers in meat products. Thus, Sanjeewa et al. (2010) evaluated the use of chickpea, pea, and wheat flours as fat replacers in bologna sausages. The flours added at 5% level increased cooking yield and decreased purge loss of the Bologna sausages, chickpea and pea also improved the instrumental texture, but only chickpea flour was associated with desirable sensory texture properties.

Similarly, Pietrasik and Janz (2010) reported that pea products (flour, fiber, and starch) added at 4% level in low-fat Bologna sausages reduced cooking and purge losses. However, only pea fiber and starch raised the texture parameters of the Bologna sausages comparable to control. Sensory acceptance was not affected by the pea starch or pea fiber addition, but it lowered with pea flour added.

Aslinah, Yusoff, and Ismail-Fitry (2018) reported that adzuki beans flour added from 3.5 to 14.2% as fat replacer lowered the calorie value of the meatballs, also adzuki flour (added at 14.2% level) raised cooking yield, hardness, and chewiness; however, taste and overall acceptance of the meatball decreased with this adzuki flour level.

The plant-based dietary fibers and gums have been applied in low-fat meat products with successful, mainly because the water holding capacity and the gelling properties that raised cooking yield, succulence and texture-related properties (Carvalho et al. 2019; Guedes-Oliveira et al. 2016; Henning, Tshalibe, and Hoffman 2016; Yousefi, Zeynali, and Alizadeh 2018).

Guedes-Oliveira et al. (2016) reported that chicken patties with apple fiber (3-4.5%) lowered the energy value, increased the cooking yield and decreased the shortening without any change in shear force and sensory acceptance. Carvalho et al. (2019) showed that hydrated wheat fiber (1.6-6.3%) added to beef burgers presented cooking loss comparable to control for all formulations, but shortening and texture parameters decreased with wheat fiber added. Abbasi et al. (2019) related the replacement of fat by water and two varieties of tragacanth gum (0.25-1%) lowered the cooking loss, extractable fat and water, and hardness values of the sausages. Protein oxidation (express as carbonyl value) reduced over storage time with 1% level of tragacanth gum. Sensory acceptance was comparable between sausages with tragacanth gum and high-fat control sausage.

Yousefi, Zeynali, and Alizadeh (2018) reported that quince seed gum (QSG) (1.2-4.8%) decreased cooking loss and TBARS values and raised firmness of low-fat hamburgers. According to the authors, QSG due to its mucilaginous jelly texture and structure can hold the water in its structure and keep more moisture during cooking, QSG also is an excellent source of caffeoylquinic acid, which is the main reason for its high antioxidant activities. Saengphol and Pirak (2018) reported that replace fat by hoary basil seed mucilage gel (HBM-98% water content) decreased the water holding capacity and hardness of chicken meat model, probably due to the high-water content in HBM gel. However, it was able to substitute 80% of pork back fat without any change of sensory perception.

Concerning the examples presented, we could conclude that several efforts have been expended to elaborating healthier low-fat meat products. Several examples of plant-based

compounds demonstrated high nutritional and technological properties to this purpose. However, regarding the plant seed mucilages use in meat products, few studies have been conducted. Seed mucilages due to their health associated aspects and significant thickening and gelling ability (Soukoulis, Gaiani, and Hoffmann 2018) may be an excellent alternative to improve the quality of the low-fat meat products.

5. Nutritional and technological properties of chia and derivatives

Chia seed (*Salvia hispanica* L.) is an oleaginous, considered a pseudocereal, annual and summery plant, belonging to the *Lamiaceae* family, native to southern Mexico and northern Guatemala (Ayerza and Coates, 2011). These seeds were used by the Aztec tribes in the early history of Mesoamerica, being one of the main staple foods used by the civilizations of Central America (Ayerza, 1995). Currently, its global production has increased, as has its popularity due to its nutritional properties, such as oil content (between 30-33%) and fatty acid composition (approximately 80% of total are unsaturated fatty acid), carbohydrates (26-41%), dietary fiber (18-30%), protein (15-25%), and minerals (4-5%) (Segura-Campos et al. 2014). Chia has been commercially grown in Argentina, Colombia, Ecuador, Peru, Bolivia, Paraguay and Australia (Busilacchi et al., 2013).

Since 2009, chia is allowed in breads up to 5% in context of the novel food regulation of the EU (EC, 2009). The commission implementing decision in January 2013 (EC, 2013) extended the use of chia as novel food ingredient, which provides further support for studies and commercial exploitation of these seeds. There is no evidence of adverse effects or allergenicity caused by whole or ground chia seeds (EFSA, 2009), thus, chia seeds and derived products are promising sources of food.

The proteins in chia seeds are characterized by a balanced content of essential amino acids and a particularly high level of the sulfur-containing amino acids methionine and cysteine. In addition, these authors found low levels of lysine and high levels of glutamic acid (Sandoval-Oliveros and Paredes-López, 2013). This seed contains protein greater than other traditional grains, like wheat, corn, rice, oats, and barley (Ayerza and Coates, 2004; Silva et al., 2017), and is therefore a promising source of bioactive peptides (Coelho et al., 2018).

Moreover, the seeds of this plant stand out due to their high concentrations of minerals, including calcium, phosphorus, and potassium, besides possessing magnesium, iron, zinc, and copper (Silva et al., 2017). The concentration of calcium in chia seed was observed to

be six times higher than that of milk, whereas the iron concentration was observed to be 2.4–6 times higher than the other sources of this mineral, as meat (Muñoz et al., 2013). However, the bioavailability of these minerals from chia has been previously reported in a few studies. Silva et al. (2019) demonstrated that the chia intake presented low calcium bioavailability regardless of the type of diet consumed, but was able to improve inflammation and the lipid profile in young Wistar rat. Besides this, the consumption of this seed increased the activity of antioxidants enzymes.

In recent years, chia seed has been highlighted, becoming increasingly important for human health and nutrition due to its high content of α-linolenic fatty acid (ALA, 18:3 n-3) (Fonte-Faria et al., 2019; Oliva et al., 2013; Chicco et al., 2009). Chia seeds have about 25-38% oil by weight, which contains the highest proportion of α-linolenic acid (above 60%), (Ixtaina et al., 2011; Ayerza, 1995). Compared to other vegetal sources of omega-3, chia oil contains a higher amount of α-linolenic than other sources such as flaxseed (52%) (Lane et al., 2016), echium oil (41.6%), or algae oil (47.7%) (Nogueira et al., 2019). Also, chia oil has significant amounts of squalene and phytosterols. Álvarez-Chávez et al. (2008) determined the phytosterols content of two Mexican varieties of chia seeds (Sinaloa and Jalisco) and found the content of individual sterols, β-sitosterol, stigmasterol, and stigmastanol, of both seeds, were superior to that of peanut, rapeseed, safflower, sesame, and sunflower unrefined oils.

Different epidemiological and clinical studies have suggested that a higher concentration of ALA is associated with a reduced risk of cardiovascular disease (Mozaffarian et al., 2005; Djousse et al., 2005). Besides may play an important role in the prevention and treatment of hypertension, diabetes, and other inflammatory and autoimmune disorders, and cancer (Simopoulos, 2002; Raygan et al., 2019; Park et al., 2019). The phytosterols and squalene present in chia oil also have beneficial effects on health, known for hypocholesterolemic and anticarcinogenic effects (Phillips et al., 2002).

Chia and its derivatives are also important sources of phenolic compounds, which are relevant dietary sources of natural antioxidants for the prevention of diseases caused by oxidative stress. Oliveira-Alves et al. (2017) reported that the main phenolic compounds found (flour and chia oil) were caffeic acid and danshensu and its derivatives, such as rosmarinic and salvianolic acids. Some of these compounds, according to these authors, were electrochemically active under the conditions of analysis and can be related to the antioxidant activity of the extracts.

The total dietary fiber (TDF) has become an essential component in the daily diet. Intake of TDF has health beneficial effects such as increased satiety, reduced risk of

cardiovascular disease and risk of colorectal cancer, decreased glycemic levels and blood cholesterol, among other benefits (Dhingra et al., 2012). The 2015-2020 Dietary Guidelines for Americans addressed the concern that most people consume inadequate amounts of dietary fiber, and thus recommendations were to choose foods that provide more fiber, such as whole grains. This guide accomplished recommendations for daily fiber consumption by age group, for example, men and women aged 19-30 years should consume 28 and 33.6 g of total fiber, respectively.

Chia seeds have considerable amounts of dietary fiber. Reyes-Caudillo, Tecante, and Valdivia-López (2008) determined the fiber content of two Mexican varieties of chia seeds and found values between 37% (Sinaloa) and 39.9% (Jalisco). Porras-Loaiza et al. (2014) evaluated chia seeds from four different regions of Mexico and found, on average, 31.1% of the fibers. These results show that environmental factors and differences in seed varieties may influence chia composition; however, significant amounts of fiber can still be considered.

The fraction corresponding to the soluble dietary fiber of the chia seed is partially exuded when it enters in contact with the water, resulting in a clear mucilaginous gel, which remains attached to the outer layers of the seed. The structural units of this polysaccharide are formed by D-xylose, α-D-glucose, and 4-0-methyl-α-D-glucuronic acid in the proportion of 2: 1: 1, respectively (Lin et al., 1994). The chia seed has 5 to 6% mucilage in its composition, which can be used as a source of soluble fibers (Reyes-Caudillo et al., 2008).

The available data on the functional properties of the chia mucilage are relatively recent and indicate that it is a polymer with thickening properties (Capitani et al., 2015; Timilsena et al., 2016). However, the potential of polysaccharide gums due to its exceptional properties has been highlighted by FAO since 1996 (Hulse, 1996). Mucilage extracted from chia seeds may become a promising ingredient for developing healthier products because of their physiological and functional properties. Among its functional properties, the most important are its high-water retention capacity, emulsifying capacity, high solubility, stabilizing agent and viscosity, besides being able to contribute to reformulations of products with low-fat content (Vázquez-Ovando et al., 2009; Goh et al., 2016). Regarding the physiological properties, it may be highlighted that chia mucilage, being a soluble food fiber, forms high viscosity gels that produce gastric distension, slower emptying of the stomach and sensation of satiety, besides to retarding the rate of absorption of nutrients from the small intestine resulting in reduced postprandial glucose levels, lowering the glycemic index (Hoad et al, 2004).

From the technological point of view, the use of dietary fiber from different botanical origins, including chia seeds and their derivatives as sources of fiber, to develop

functional meat products is a promising trend. Fibers have multifunctional properties, among which we can mention: improvement in water retention capacity and texture, stabilization of fat in emulsified products, exert a mimetic behavior of fat in products that have reduced composition, among others. In addition to being exploited as a form of nutritional enrichment of meat products (Fernández-Ginés et al., 2005). The defatted flour of chia, for example, has contents of up to 40% of fiber. This fiber content is higher than quinoa, flaxseed, and amaranth, even higher compared to other dehydrated products (Muñoz et al., 2013).

6. Technological applications of chia and derivatives in meat products

Chia seed is widely used in the food industry as an ingredient for the development of healthy and dietetic foods due to its high fiber content, besides its technological properties (Vázquez-Ovando et al., 2009; Ding et al., 2018; Capitani et al., 2015). According to Salgado-Cruz et al. (2013), the chia form hydrated mucilage in water that can be used in the food industry due of their excellent physicochemical properties.

The technological properties provided by dietary fibers depend greatly on the properties of hydration (solubility, swelling, gelling, viscosity, absorption and water-holding capacity) (Borderías, Sánchez-Alonso, and Pérez-Mateos, 2005; de Falco, Amato, and Lanzotti, 2017) besides density and surface characteristics, particle size, cationic exchange capacity and organic molecule adsorption capacity (Vázquez-Ovando et al., 2009). Chia seed can act as a stabilizer, texturizing, emulsifying, gelling and suspending agent, increases fat and water retention among other properties (Vázquez-Ovando et al., 2009; Elleuch et al., 2011; Reyes-Caudillo et al., 2008). However, the technological properties of the chia seed are influenced by several factors such as environmental conditions, genetic modifications and agricultural practice, geographic origin and type of extraction of isolates or mucilage (de Falco et al., 2017; Ixtaina et al., 2011).

The chia gum can be extracted from the fibrous fraction by treating the seeds with water (Capitani et al., 2015). According to Capitani, et al., (2012), there is more dietary fiber soluble and insoluble in the fibrous fractions. In comparison to other sources of fiber such as wheat and soybean, the fiber rich fraction of chia has greater emulsifying activity, water retention and absorption (Vázquez-Ovando et al., 2009). To obtain the largest amount of mucilage, Muñoz et al., (2012) analyzed the extraction under different conditions of seed/water ratio, temperature and pH. The highest yield was achieved at 1:40 (seed/water), 80 °C and pH

8. Chia gum is stable at high temperatures (more than 244 °C) (Timilsena et al., 2016) while the oil is not recommended for cooking or frying due to instability and degradation of unsaturated fatty acids (Guimarães-Inácio, et al., 2018; Villanueva, et al., 2017).

Chia have been used as whole seeds, oil and flour in meat products. In general, chia seeds are grounded, dried or soaked in water before adding in meat products (Zettel and Hitzmann, 2018), being these derivatives widely available commercially. Different strategies of reformulation with chia in meat products are reported in the literature, but the most common forms of the addition are directly in the formulation (seed or flour) or incorporated through of emulsions, emulsion gels or hydrogels. Besides, many studies using chia oil, incorporated through microcapsules or in emulsions, according to Table 2.

Table 2. Application of chia seed or derivatives in meat products and nutritional, technological and sensory benefits, and main limitations found.

Chia seed or derivatives	Incorporation strategy	Meat product	Technological and sensory benefits	Nutritional benefits	Limitations found	References
Chia flour	1) chia oil-in-water emulsion with olive oil or 2) emulsion gel with chia flour, sodium alginate and olive oil	Frankurters	Reduction of purge in all samples; Frankfurter were judged acceptable; good statility to oxidation and safety during storage.	Fat and energy content reduction (>26%); chia increased total dietary fibre and linolenic acid	Lightness and redness of frankfurters were affected by the presence of chia	Pintado et al. (2016)
Chia flour	In various concentrations and direct addition	Chicken nuggets	The oil absorption, weight gain of the coating and cooking yield were not affected by the incorporation of chia flour. Nuggets (up to 10% of chia flour) were considered acceptable.	Increase of polyunsaturated fatty acids and dietary fiber	Objective color and texture parameters were affected	Barros et al. (2018)
Chia seed	In various concentrations and in combination with carrageenan (CAR)	Ham	1% chia seed decreased oxidation of lipids and proteins; the chia and CAR combination increased yield on processing.	Reduction of fat, higher concentration of polyphenols	Control group demonstrated the best scores for odor, color, texture, flavor, and overall acceptance	Ding et al. (2018)
Chia oil	Microparticles containing chia (CO) and linseed (LO) oils obtained by external ionic gelation.	Hamburgers	The lipid reformulation did not affect hardness and improved technological properties, such as cooking loss and fat retention.	Reduction of fat, healthier PUFA/SFA and n-6/n-3 ratios, and lower atherogenicity and thrombogenicity indices.	Higher lipid oxidation and a lower sensory quality compared to the other treatments.	Heck et al. (2017)

Table 2. Continuation

Chia seed or derivatives	Incorporation strategy	Meat product	Technological and sensory benefits	Nutritional benefits	Limitations found	References
Seeds, flour and a coproduct from cold-press oil extraction	3% and direct addition	Frankfurters	Better resistance to oxidation; lower residual nitrite levels; no effect on microbiological safety; did not modify the redness of frankfurters.	Increase of dietary fiber	Lowest L* values and the highest b* values (chia coproduct)	Fernández-López et al. (2019)
Chia oil	Hydrogelled emulsion (HE) from chia and linseed oils.	Hamburgers	No changes were observed for the moisture retention, diameter reduction, and cooking loss of the treatments.	Healthier fatty acid profile with health claims	Increase of hardness and a significant color difference (ΔE)	Heck et al. (2019)
Chia flour	Emulsion gel with chia flour, sodium alginate and olive oil (CEG)	Fresh sausage	Cooking loss was lower; all sausages were judged acceptable.	CEG improved MUFA and PUFA contents, increased of dietary fiber; reduction of fat, increase of amino acids (aspartic acid, serine, glutamic acid)	Changes in sensory characteristics of the products; microbial count was affected by the use of CEG after 13 days.	Pintado et al. (2018)
Chia flour	Emulsion gel with chia flour, soy protein isolate, soybean oil	Bologna sausage	Higher stability of the meat batters; low lipid oxidation level; chia flour addition affected the protein matrix and a more compact structure was observed.	Higher amount of polyunsaturated fatty acids, increased the omega 3 content, and reduced saturated fat up to 41%.	Modification of color and texture parameters	Paglarini et al. (2019)

Although chia mucilage has several technological properties (Muñoz et al., 2012), no fat substitution studies have yet been reported for this component in meat products, which may be an excellent opportunity for future researches.

Considering the necessity reformulating meat products, particularly regarding fat reduction, recent studies with chia seed and flour in meat products show that the most common nutritional benefits found are increased dietary fiber content, reduced fat, and a better fatty acid profile. The most reported technological properties are the reduction of purge or cooking losses and oxidation stability, possibly due to the polyphenols present (Ding et al., 2018; Souza et al. 2015, Barros et al., 2018). The technological limitations found most frequently in the studies of reformulation with seed and chia flour refer mainly to changes in texture, color, and decreased sensory acceptance (Ding et al., 2018; Fernández-López et al., 2019).

Another way of incorporating chia flour widely used in reformulation studies of meat products is through the previous elaboration of emulsion gels or hydrogels, with the addition of healthier oils along with chia flour and gelling agents (Pintado et al. 2016; Paglarini et al., 2019). The addition of chia flour in the form of emulsion gels shows less interference in the sensory acceptance and better stability of the emulsified meat products when comparing with the flour directly added in the products (Pintado et al., 2015; Herrero et al., 2017).

6.1 Rheological behavior of chia mucilage and potential of technological application

The desired texture is commonly a foremost feature to be reached in ingredient development and elaboration of new structures/systems envisioning food applications. Therefore, rheological measurement appears as a tool for physical characterization along all chain of production including the raw material, the intermediate product during manufacturing, and for the final product (Tabilo-Munizaga and Barbosa-Cánovas, 2005). Thus, rheological characterization can be useful considering food processing steps (i.e.pumping), handling and the food acceptance by consumers (Fischer and Windhab, 2011).

Fluid gels offer a number of tuneable material properties, and as such, they have potential use in diverse applications including the noble function as fat replacer in food formulations (Garrec and Norton, 2012; Fernandes and Salas-Mellado, 2017). The rheological properties of these soft materials - as chia mucilage - can be tailored in a subtle manner to meet the requirements for desired technological application.

It is reported that rheological properties of hydrocolloids in solution can be dependent of a number of variables such as concentration of the component, shear rate and time, temperature, pressure, ionic strength (salt or sucrose addition) and pH (Karazhiyan et al., 2009; Capitani et al., 2015). Chia mucilage solution has shown attractive technological applications such as thickener agent, gel former and chelator property (Capitani, et al., 2012).

Chia-based gels have been used as fat replacer since when hydrated mucilage can develop viscosity (Vázquez-Ovando et al., 2009). It has been used to replace eggs in cakes due to its high content of healthy fats without compromising the sensory quality of these products (Borneo et al., 2010). In addition, meat products (i.e. sausages) have been reformulated using chia flour in emulsion gels (Pintado et al., 2018). Chia based gel also presented potential application, being able to replace all emulsifiers and stabilizers used in ice cream with quality maintenance (Capitani, et al., 2012).

Rheology of chia mucilage may explain such interesting features explored behind the technological application of chia as texture modifier. When it comes to flow behaviour, chia mucilage exhibit shear thinning behaviour (1 to 5% w/w) with behaviour index (n) lower than 1 (García-Salcedo et al, 2018). It means that the complex viscosity (η^*) decreases as function of the frequency, and therefore it has dependence on the shear rate. Other published studies have been demonstrated that power-law model ($\tau = k.\gamma^n$ where τ =strain (Pa); k=consistence index; n=behavior index; and γ= shear rate (1/s)) is the best model for describing the flow behaviour of the food hydrocolloids (Koocheki, Taherian, and Bostan, 2013; Capitani et al., 2015; García-Salcedo et al., 2018). The pseudoplastic feature is related to the randomly positioned chains of the biopolymer molecules that are prone to align following the direction of the flow at higher shear rates, causing less interaction among adjacent polymer chains (lower resistance), thus lowering viscosity (Koocheki et al., 2013). However, if an overall higher viscosity is required it is possible to reach it by increasing the mucilage concentration in the dispersion (Capitani et al., 2015). In this case, the higher content of total solids may cause a greater restriction of intermolecular motion as response of hydrodynamic forces and the formation of an interfacial film (Maskan and Gogus, 2000). Moreover, it is reported that even at low concentration of polysaccharide (0.05% w/w) the pseudoplasticity of the sample persisted (Capitani et al., 2015; Goh et al., 2016; García-Salcedo, 2018).

The methods of extraction are also an important variable that can alters mechanical behaviour in the case of chia mucilage. A study comparing hot and cold extraction showed that shear-thinning behaviour was most notable in the dispersions obtained by cold extraction and

with high concentrations. Moreover, the gum obtained using cold approach was more thixotropic than the ones obtained by conventional hot extraction (Tavares et al., 2018).

In terms of oscillatory, rheological behaviour chia mucilage possess a resembling gel-like structure where viscoelastic dominate over storage properties ($G' > G''$) forming a gel-like structure (Capitani et al., 2015). The gel-like behaviour in anionic polysaccharides is well supported by the formation of ionic clusters within the three-dimensional matrix (Timilsena et al., 2015). For chia mucilage, the synergistic interactions between fibers and proteins are responsible by viscosity increasing within the suspensions (García-Salcedo et al., 2018). Similar to the flow behaviour, the mucilage concentration affects the rheological properties during oscillation frequencies at a constant oscillation amplitude and temperature. The higher concentration of mucilage it implies in a greater elastic modulus (G'). Therefore, frequency dependence of mechanical spectrum is mainly dictated by the concentration of chia mucilage. Eventually at low concentration, it is possible to follow the crossover between loss and storage moduli. On the other hand, a behaviour independent of observation time is reported at higher concentration (Capitani et al., 2015).

Interestingly mucilage did not exhibit a dependency on temperature which can open the opportunity to apply it in a wide range of food with different temperatures. A study performed by García-Salcedo and collaborators (2018) compared rheological properties of chia flour and mucilage. Although it was observed that mucilage produced low viscosity systems compared with the chia flour, its behaviour was not dependent on temperature. At different concentrations, when the mucilage solution was cooled after a temperature ramp (heating), it recovered its initial viscosity, which is related to mucilage composition mainly formed by soluble fibbers.

These potential characteristics of chia mucilage encourage its application in derived systems such as oil-in-water emulsion gels stabilized with mucilage (Capitani et al., 2016). It has been shown that chia mucilage dispersions present viscoelastic properties at the vicinity of the interface oil-in-water in emulsions. Thus, there is the stratification of the mucilage material surrounding the oily phase, providing to the mucilage emulsification and stabilization abilities (Avila-de la Rosa et al., 2015). The emulsions with chia mucilage presented viscosity proportional to the mucilage concentration. It could be explained by the larger proportion of high-molecular-weight molecules in the aqueous phase that may promote a greater resistance to flow by inhibiting the movement of the droplets and frequency of collision, which might favour the formation of a three-dimensional structure network that can prevent creaming (Capitani et al., 2016).

7. Satiety properties in the development of new fat substitutes

Obesity is a global problem which increased prevalence can cause and aggravate several diseases with a high mortality rate, such as cardiovascular diseases (Börschel and Schnabel, 2019). Over the past 40 years, according to the World Health Organization, obesity has more than doubled worldwide (WHO, 2016). Dietary fat is an important determinant for energy density of the diet and thereby for energy intake (Astrup, 2002), however, its role in the increasing prevalence of obesity is currently discussed (Sundfør et al., 2019; Yeomans, 2017).

In this away, a common strategy for reducing the risk of overweight and obesity has been to reduce average energy intake by lowering fat intake. An additional strategy could be the development of foods with high satiety, which is based in the understanding how to feel satisfied with fewer calories (Blundell et al., 2010). Although one of the constant concerns of the food industry has been to produce and provide safe food to consumers, the nutritional and caloric composition is becoming equally important (Lundin, Golding, and Wooster, 2008). The balance between ingestion and energy consumption is critical to maintaining a healthy life, and sophisticated physiological mechanisms are designed to do this, including appetite control (Benelam, 2009). Satiation and satiety are central concepts for understanding appetite control, and both are related to inhibition of consumption. Satiation is related to decreased appetite during food intake and can be quantified by the duration or size of the meal. Satiety begins after the consumption of the food, being the feeling of fullness that persists after eating, suppressing the additional consumption of food (Bellisle et al., 2012).

Factors affecting satiety and satiation have been characterized in some studies as being divided into sensory, cognitive, post-ingestion and post-absorption stages, commonly called the “satiety cascade”. Initially, the factors that will influence are sensory and cognitive, more related to satiation, including expectations about the food to be consumed, related to aspects such as taste, texture and aroma or associations with previous experiences that may arise. Once the food or drink reaches the stomach, post-ingestion factors begin to take effect. Initially, the distension of the stomach sends signals to the brain, initiating the sensation of satiety. As the digestion process continues in the intestines, hormones that promote satiety are released at this location. In the post-absorption stage, the nutrients themselves are detected by specialized receptors in various parts of the body, including the brain, providing information on nutritional status which will also affect satiety (Blundell, 2006).

According to Holt et al. (1995) types of foods composed of different macronutrients (lipids, proteins, and carbohydrates), even if isoenergetic, will also produce distinct physiological effects that will reflect varied effects on satiety, thermogenesis and nutrient storage in the body. About to dietary fat and appetite control, studies have shown that high-fat foods result in fragile effects on satiation and satiety (Blundell and MacDiarmid, 1997). These foods promote a positive energy balance, since they have a high energy density, besides having high palatability (Rolls, 2000). However, the fat consumed seems to generate satiety signals that appear more slowly, that is, a large amount of energy in the form of fats can be consumed before the signs of satiety induced by this macronutrient become fully active (Lawton, Burley, and Blundell, 1993; Lawton and Blundell 1998).

Researches that compares the satiating effects of macromolecules constituting foods are conclusive: protein is the most effective in prolonging satiety perception, followed by carbohydrates and then lipids (Anderson and Moore, 2004; Veldhorst et al., 2008; Solah et al., 2010). According to Veldhorst et al. (2008), several mechanisms may contribute to the satiety induced by protein intake, such as the increased concentration of satiety hormones, energy expenditure, contents of metabolites, that is, amino acids and in the gluconeogenesis process. Proteins have unique characteristics related to their origin, amino acid content, and absorption kinetics. Proteins from different sources are considered to have different metabolic effects (Gilbert et al., 2011), and there is some evidence that various sources of protein differ in their capacity for satiety; however, there are contradictory studies and satiety promotion mechanisms are still not entirely elucidated (Bendtsen et al., 2013, Gilbert et al., 2011).

Another macronutrient with an essential role in modulating satiety is nondigestible carbohydrates, such as dietary fibers (Brennan, 2005). Dietary fiber has been defined such as primarily as the storage and cell wall polysaccharides of plants that cannot be hydrolyzed by human digestive enzymes (Marlett, McBurney, & Slavin, 2002) and depending on their behavior in aqueous solutions it is classified in soluble such as mucilages, pectins, gums, etc. and insoluble such as cellulose, lignin between others (Dhingra et al., 2012). Dietary fibers can induce satiety in several ways: they increase chewing time and thereby increase the secretion of saliva, gastric juice, and satiety hormones; decrease the rate of absorption of nutrients in the small intestine and also by the modulation of blood glucose (Bellissimo and Akhavan, 2015; Brennan, 2005).

Increased gastric distension is another factor with an impact on fiber-induced satiety. Fibers absorb large amounts of water, adding volume and weight, producing foods with higher volume and low energy density that trigger gastric and postgastric mechanisms. Gastric

distension during food intake activates nerve fibers, called vagal afferents, which send signals from the stomach to the brain and result in the perception of satiety (Slavin, 2005). However, efficacy varies widely between the type of fiber (soluble vs. insoluble), the amount and mechanisms of action in the body (Slavin and Green, 2007). High intrinsic viscosity fibers such as mucilages, pectin, β-glucan, guar gum, and alginate are more frequently correlated with increased satiety and reductions in energy consumption, possibly due to their ability to absorb water and the formation of gels within the gastrointestinal system (Li and Nie, 2016). According to Vuksan et al. (2017), chia seeds appears to have the ability to convert glucose into a slow-release carbohydrate and affect satiety to a greater extent than flax, possibly due to the higher fiber viscosity.

Based on the evidence discussed above, regarding the potential satiation of macronutrients, it is observed that food products with high protein content and that may still have the addition of dietary fibers have great satiety capacity and may be the basis for the development of these types of products, with lower energy content and more satiating. In this context, meat products, which traditionally have high-fat contents (20 to 35%) (Feiner, 2006), have been the focus of reformulation studies to reduce this lipid content and substitution by several compounds. Such as different soluble and insoluble dietary fibers, with significant technological and sensorial advances (Olmedilla-Alonso, et al., 2013).

Choe et al. (2013) evaluated the reduction of 50% of fats in Frankurters and the addition of 20% of wheat fiber mixture and had satisfactory results with more stable emulsions without differences in the sensorial attributes between the fiber samples and the control. Álvarez and Barbut (2013) demonstrated that the appropriate addition of inulin and β-glucan blends could compensate for some changes in texture caused by reducing fat in cooked meat batters, besides reducing cooking losses. Thus, numerous studies, in addition to the cited examples, demonstrated several benefits of dietary fibers as fat substitutes. Dietary fibers are promising fat substitutes, since many of these functional ingredients can mimic the pleasant palatability of fats without, however, substantially increasing caloric intake. Besides, texture also influences satiety. Products with a denser structure and greater chewiness (such as some meat products reformulated with fibers) have a higher satiety capacity than less dense or liquid foods (Camire and Blackmore, 2007). However, very little has been elucidated in these new fat substitutes in the meat matrix concerning the physiological effects, notably for satiety and for the transformations that occur in the gastrointestinal tract and its main impacts.

Some studies have worked with the perceptions of satiety involving meat products with the reduction of fats and the addition of dietary fiber, but they are still scarce, and the

results are conflicting. Vulhom et al. (2014) demonstrated that low-fat sausages added with rye and wheat bran promoted greater satiety and lowered prospective consumption when compared to sausages added with refined wheat flour. On the other hand, Kehlet et al. (2017) did not observe any effect on satiety when evaluating pea fiber and rye bran in meatballs, although there was an improvement in nutritional composition. Archer et al. (2004) concluded that the inulin and lupin-kernel fibers appear to have potential fat replacers in meat products and for reducing fat and energy intake in men. Finally, the most recent work by Carvalho et al. (2019) demonstrated that the partial replacement of fat by hydrated wheat fiber in beef burgers decreased caloric value without reducing the feeling of satiety after consumption.

Scientific advances in the evaluation of fat substitutes in meat products demonstrate many essential explanations from a technological, sensorial and microstructural point of view, but research and the posture of society, including consumers, are a constant evolution. It is verified that new questions are being raised, after all, before all the reformulations proposed and studied, how would be behavior in the organism of these products after ingestion? It should be taken into account that after changing the matrix, for example, of a meat product, another type of post-ingestion behavior is expected about digestibility and perceptions of appetite sensations. What are the expected interactions, absorptions, and behaviors of these macronutrients? Very little is known about all these questions, and again, it becomes essential the role of researchers in the understanding and greater elucidation of the physiological effects of these proposed fat reduction substitutes.

7.1 Digestibility as a relevant factor in fat substitutes

The understanding of the interactions between food components at a physiological level can be advantageous when developing reformulated products with functional aspects. To know how the structure of food can modulate digestion kinetics and/or macronutrient digestibility has, therefore, become a critical issue that can lead to more effective ways of maintaining a healthy diet (Norton et al., 2014). Meat and meat products are an excellent source of proteins for humans, with well-balanced in amino acids and contain all the essential amino acids that humans cannot synthesize. Nevertheless, meat processing (comminution, cooking, salting, curing, and storage) can affect the structure of a protein, and consequently, the digestibility of this nutrient (La Pomélie et al. 2018)

An understanding of the molecular interaction and the basic mechanisms affecting digestive behavior in reformulated meat products with fat reduction and addition of functional compounds (i.e., dietary fiber) are required for an enhancement of the technology used. In addition, it is important to have physiological effects of nutrient absorption similar to those obtained with conventional meat products (Baugreet et al., 2019), or depending on the reformulation, with a higher perception of satiety. The interactions between the macronutrients (such as dietary fiber, protein and lipids) will influence not only the physiological responses and the nutritional composition but also the structure of the product, its distribution in the food, particle size, and shape, which in turn will determine its texture, palatability and moisture perception (Lundin et al., 2008). Rheology and texture may also play an important role in the digestive process and subsequent metabolic response (Robertson, 2006).

The rheological properties of digested food within the gastrointestinal tract are crucial in determining motility and transit time. Gastric emptying and nutrient absorption are strongly delayed when chyme viscosity is increased (e.g., soluble dietary fibers) (Slavin, 2005). The importance of soluble fiber consumption comes from its fermentation in the large intestine. Soluble fiber (water soluble) by passes the digestion of the small intestine and is readily fermented by the microbiota in the large intestine, while insoluble fiber (not water soluble) have very limited fermentation (Brenan, 2005). Due to this increase in viscosity, soluble fibers may exert beneficial metabolic effects, inhibiting the absorption of nutrients and binding of bile acids, as well as a decrease in the postprandial glycemic response, reduction of serum and liver cholesterol (Graaf et al., 2004).

Although several studies report the increased viscosity in the digestive tract provided by soluble fibers, Tamargo et al. (2018) observed that chia mucilage (at the concentrations used in this study, 0.3, 0.5 and 0.8%) did not affect the physical properties of the intestine (viscosity) but could affect the colonic microbial growth. On the other hand, Lazaro et al., (2018) evaluated the chia mucilage (at same concentrations as in the previous study) through in vitro micro-digestion and observed only a slight reduction of viscosity during the digestion process, and suggested that the mucilage could maintain its structure in the food matrix and be used to develop structured foods.

The elucidation of microstructural behavior through gastrointestinal simulations of more complex food matrices such as reformulated meat products is still an area of study with few studies, more recent. One of them is the study developed by Baugreet et al. (2019) that included lentil flour and plant proteins in restructured meat model systems and evaluated the microstructural changes of the products during the in vitro digestion phases. These authors

observed that the enzymatic mechanism varied among all samples, the rupture of the alimentary matrix was unequal and did not appear to have been affected by pepsin in the same way between the products.

Lin et al. (2019) found a decrease in the digestibility of surimi products with increasing levels of soluble and insoluble dietary fiber. These studies demonstrate that there are still many interactions between macronutrients involving the process of enzymatic and mechanical degradation of digestion that need further understanding. Interdisciplinary researches interconnecting the technological area and the study of gastrointestinal physiology, together with the improvement of in vitro simulation techniques, more adjusted and similar to the organism, will support the development of healthier and bioavailable reformulated products (Lundin et al., 2008).

8. Conclusion

The challenges in replacing fat in meat products are continually changing with new insights in this area of research. Many natural compounds have been used with relative success in meat products with improved texture, seeking to approach more of the ideal behavior provided by animal fat. Chia mucilage and other mucilages, as well as compounds added with recent technologies in meat products (organogels, emulsion gels), still constitute an ample field of research to be explored under a new point of view, which also aims at better physiological properties with greater benefits to the health of consumers. Chia mucilage has enormous potential to be exploited as an innovative fat substitute, due to its inherent ability to gel formation, emulsion stabilization, nutritional properties, and be a thickening agent. Moreover, it has characteristics that can be very beneficial to the organism, since it is a soluble fiber, for this reason, can promote greater satiety to products with fat reduction. Thus, new sources of functional compounds will be continually explored, but not only with a viability and technology focus, but focused on the real functionality of these products, that is, if it can confirm the better functioning of the organism, either increasing the absorption of nutrients by the body, or, for example, improving the perception of satiety. Chia mucilage is a promising ingredient that can meet such expectations.

Declarations of interest

The authors declare that they have no conflict of interest.

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CAPÍTULO 2

CHIA (*SALVIA HISPANICA* L.) MUCILAGE AS A NEW FAT SUBSTITUTE IN EMULSIFIED MEAT PRODUCTS: TECHNOLOGICAL, PHYSICOCHEMICAL AND RHEOLOGICAL CHARACTERIZATION

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Chia (*Salvia hispanica* L.) mucilage as a new fat substitute in emulsified meat products: technological, physicochemical and rheological characterization

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Abstract

The objective of the study was to characterize chia mucilage (MC) and evaluate the rheological properties of the gels performed by this functional ingredient comparing their individual effects when added in emulsified meat system models taking in account their healthy properties. Three different concentrations of chia mucilage gels (MCGs), 15%, 20%, and 25%, and two levels of MC 2.5%, and 5.0%, were applied aiming to substitute 50% of pork back fat in meat model systems. It was observed that the rheological behavior of mucilage was viscoelastic with a dominant storage modulus (G' > G") forming a structure type gel. The values of tan δ ranged between 0.37 and 0.40, which could indicate a weak elastic gel-like behavior in the frequency range studied. The mechanical properties of MCGs were preserved after thermal stress. The meat emulsion stability was improved with the addition MCG. All formulations with 5% MC were characterized by increased hardness, decreased elasticity and cohesiveness values and significant differences ($P < 0.05$) were found between these samples and the control (FC1). Chia mucilage thus shows potential as a substitute saturated fat in emulsified meat products with improved technological characteristics with additional healthier claims.

Keywords: fat replacement; meat products; chia mucilage; *Salvia hispanica* L.; rheology

1. Introduction

Chia (*Salvia hispanica* L.) is a summer annual herbaceous plant belonging to the *Lamiaceae* family, which is native to southern Mexico and northern Guatemala. Due to its high nutritional value and properties, there has been an increasing interest in studying chia and chia-derived products. Chia seeds contain a considerable amount of dietary fiber, antioxidants (including phenolic compounds) (Reyes-Caudillo, Tecante, & Valdivia-López, 2008), elevated content proteins with a balanced proportions of essential amino acids (Sandoval-Oliveros & Paredes-López, 2012), and are rich in polyunsaturated fatty acids, especially linolenic acid (Ding, Lin, Lin, Yang, Yu, Chen, et al., 2018). In 2009, the European Parliament and the European Council authorized the placing chia seed on the market as novel food ingredient under Regulation Nº 258/97, which provides support for studies and commercial use of these seeds.

When chia seeds come in contact with water, a clear mucilaginous gel is exuded and a transparent capsule surrounding the seed quickly forms. This mucilage is located in the outer three layers of the seed coat prior to exudation (Muñoz, Cobos, Diaz & Aguilera, 2012).

The mucilage is an anionic heteropolysaccharide consisting of xylose and glucose as the main sugars, with a xylose-to-glucose ratio of approximately 2:1. Additionally, chia mucilage contains significant amount of uronic acid (glucuronic acid and galacturonic acid) and two other neutral sugars, arabinose and galactose (Lin, Daniel & Whistler, 1994). Due to its structure, chia mucilage (MC) acts like a soluble fiber and is known to have exceptional water holding, gel-forming, shear-thinning, and emulsion-stabilizing properties (Muñoz et al., 2012; Timilsena, Adhikari, Kasapis, & Adhikari, 2016; García-Salcedo, Torres-Vargas, Real, Contreras-Jiménez & Rodriguez-García, 2018; Capitani, Nolasco & Tomás, 2016). Moreover, due to these characteristics, chia mucilage is a promising substitute for fat during food production, as reported in several studies on breads, cakes, and pastes (Fernandes & Salas-Mellado, 2017; Menga, Amato, Phillips, Angelino, Morreale & Fares, 2017). However, in meat products, which traditionally have high levels of fat, the use of mucilage as a fat substitute has yet to be studied.

Meat and processed meat products are often perceived as unhealthy by consumers due to high levels of sodium, additives, and saturated fat and a lack of fiber. For example, emulsified meat products like Bologna sausage can, traditionally, contain 20–35% fat and 2.2–2.5% salt (Feiner, 2006). Consumption of processed meat has also been associated with a higher incidence of chronic cardiometabolic diseases and diabetes mellitus (Micha, Wallace, & Mozaffarian, 2010). Reformulated meat products therefore represent a potential opportunity for the meat industry to improve this perception (Grasso, Brunton, Lyng, Lalor & Monahan, 2014). Fat content has an essential effect on characteristics such as texture, flavor, and stabilization of meat emulsions, and therefore, cannot be removed without a suitable substitute (Jiménez-Colmenero, 2000). Fat substitution is a great challenge and MC, because of its function and properties in other foods, should be investigated as a potential substitute for fat in meat products.

The viscoelastic behavior and the thickening properties of hydrocolloids such as MC can be affected by several factors including temperature, shear rate, and concentration (Capitani, Corzo-Rios, Chel-Guerrero, Bentacur-Ancona, Nolasco & Tomás, 2015). Such factors become very important when using these gels to replace fat and modify the texture of meat products, therefore their interactions with the meat matrix must be evaluated. The objective of the present study was to characterize chia mucilage and evaluate the rheological properties of chia mucilage gels (MCGs), including investigating the effects of conditions such as mucilage concentration and temperature. We also investigated the effects on meat model system of substituting 50% of fat with MCGs on their technological characteristics.

2. Materials and methods

2.1 Materials

Chia (*Salvia hispanica* L.) seeds were purchased from Cereal Prime (São Paulo, Brazil). Sodium tripolyphosphate, sodium erythorbate, and sodium nitrite were kindly donated by Kerry do Brasil Ltda (Campinas, Brazil). The reagents used in this study were of analytical grade. Pork (*M. longissimus dorsi*; 69.5% moisture, 10% lipids, 18% protein, and 1.5% ash) and pork back fat (13% moisture, 81.3% lipids, 5.21% protein, 0.49% ash) were obtained from a local market in Campinas, Brazil. After removing apparent fat and aponeurosis, meat and fat were ground in a 5.0 mm disks and frozen at −18°C for no more than 7 days until use.

2.2 Extraction of chia (Salvia hispanica L.) mucilage

Chia mucilage (MC) was obtained using procedures from Coorey, Tjoe & Jayasena (2014) and Felisberto, Wahanik, Gomes-Ruffi, Clerici, Chang & Steel (2015) with some modification. Whole chia seeds were soaked in water (1:25 w/v) for 3 h at 60 ºC using an electric cooker with automatic stirring in order to induce the mucilage exudation. The extracted mucilage was separated from the seeds using a 35/ CM-876 finisher pulper with a stainless-steel wire mesh with 0.26 mm apertures (FMC do Brasil Indústria e Comércio Ltda, Araraquara, Brazil). The aqueous suspension containing the mucilage was dried in an LP 820 freeze-drier (São Paulo, Brazil) and ground using a food processor (GM 200, Retsch, Germany) to obtain a fine powder. The MC was packaged in hermetically sealed metalized packaging.

2.3 Physicochemical and rheological characterization of chia mucilage

2.3.1 Chemical composition

The moisture, protein, lipid, and ash content of the lyophilized chia mucilage was determined following the Official Methods of Analysis of AOAC International (AOAC, 2012). The carbohydrate level was obtained by difference of the other compounds. Total dietary fiber, soluble dietary fiber, and insoluble dietary fiber were determined using official method 991.43 (AOAC, 2012). All measurements performed in triplicate.

2.3.2 Fatty acid profile

To determine the fatty acid profile, lipids were extracted following Bligh & Dyer (1959) and esterification carried out according to Hartman & Lago (1973). The methyl esters of fatty acids were separated according to the Ce-66 method (AOCS, 2009). Samples were analyzed in CGC Agilent 6850 Series GC Capillary Gas Chromatograph equipped with DB-23 AGILENT (50% cyanopropyl-methylpolysiloxane) capillary column 60 m, Ø int: 0.25 mm, 0.25 μm film. Fatty acid composition was determined by comparing peak retention times with those fatty acid standards. The analysis was performed in duplicate. The atherogenic index (AI) and thrombogenic index (TI) were calculated according to Ulbricht & Southgate (1991) as a ratio between some SFA and unsaturated fatty acids.

2.3.3 Rheological measurements

Dispersions with either 15%, 20%, or 25% w/v MC were prepared by hydrating dried mucilage in deionized water for 30 min at room temperature (25 ºC) to form chia mucilage gels (MCGs). Dispersions were then left overnight at 4°C to ensure a complete hydration prior to the rheological measurements. Concentrations of MCGs were determined in preliminary tests before application in meat products. Viscoelastic behavior of MCG was investigated by small amplitude oscillatory measurements using a stress-controlled rheometer Physica MCR 301 (Anton Paar, Graz, Austria) equipped with a Peltier system and a water bath (Julabo, Seelbach, Germany) for temperature control. The experiment was performed using cone-plate geometry (50 mm, 2° angle, truncation 208 μm). First, a strain sweep was performed by logarithmically increasing the strain from 0.01 to 10% at a frequency of 1.0 Hz to identify the linear viscoelastic region (LVR) of samples. Frequency sweeps of 0.01–10 Hz were subsequently performed at 5°C and a strain value within the LVR.

Sample behavior after either heating and a frequency sweep at 72°C or heating followed by cooling (72°C to 5°C) and a frequency sweep at 5°C were carried out to mimic thermal treatment during emulsified meat product production. Rheological measurements were performed using of protocols: (i) temperature ramp from 5°C to 72°C (5°C·min^{-1}); (ii) 72°C for 5 min; (iii) frequency sweep at 72°C or (i) temperature ramp from 5°C until 72°C (5°C·min^{-1}); (ii) 72ºC for 5 min; (iii) temperature ramp from 72°C to 5°C (5°C·min-1); (iv) 5°C for 5 min; (v) frequency sweep at 5°C. The elastic component (G'), viscous component (G"), and tan delta

(tan δ) were recorded. All measurements were performed in triplicate and samples covered with silicone oil to prevent water loss.

2.3.4 Attenuated Total Reflectance (ATR)-FTIR spectroscopy analysis

The infrared spectra of powdered samples were recorded using a Perkin-Elmer Spectrum TM 400 spectrometer (Perkin Elmer Inc., Madrid, Spain) in mid-IR mode, equipped with an ATR (attenuated total reflectance) sampling device containing diamond/ZnSe crystal. Measurements were performed at room temperature (25 ºC) using approximately 25 mg of MC, which was placed on the surface of the ATR crystal and gently pressed with a flat-tip plunger. The spectra were scanned in the 4000–650 cm^{-1} wave number range with a scan speed of 0.20 cm/s and 8 accumulations at a resolution of 4 cm^{-1}. Ten measurements were taken and summed to obtain the total spectrum (80 accumulations). A background spectrum was generated using the same instrument conditions before each measurement.

Spectra were acquired with the Spectrum software version 6.3.2 and spectral data were processed with the Grams/AI version 9.1 (Thermo Electron Corporation, Waltham, MA) software.

2.4 Experimental design and preparation of meat model systems

Meat emulsion model systems were evaluated with three different concentrations of MCG, 15%, 20%, or 25% and two concentrations and two final concentrations in the product of MC, 2.5% or 5.0%. For MCG incorporation, lyophilized mucilage was rehydrated in water and allowed to stand overnight before processing at 4°C. Six treatments with 50% of fat reduction and with different amounts of MCG were made along with two control treatments, FC1 and FC2, containing 20% or 10% fat, respectively, without MCG, as described in Table 1. Each meat emulsion also contained the following additives: 2% sodium chloride, 0.015% sodium nitrite, 0.05% sodium erythorbate, and 0.25% sodium tripolyphosphate.

Meat model systems were prepared according to Paglarini, Furtado, Honório, Mokarzel, Vidal, Ribeiro, et al., (2019). Products were then cooled in ice and stored at 4°C no more than 3 days until analysis.

Table 1

Description of treatments (%, w/w) of meat model systems with chia mucilage gels (MCG) in different concentrations.

Ingredients	Treatments (%)							
	FC1	FC2	MCG15-2.5%	MCG15-5%	MCG20-2.5%	MCG20-5%	MCG25-2.5%	MCG25-5%
Pork	54.66	54.66	54.66	54.66	54.66	54.66	54.66	54.66
Pork back fat	20	10	10	10	10	10	10	10
Chia mucilage powder- total (MC)	0	0	2.5	5	2.5	5	2.5	5
Chia mucilage gel (MCG)	0	0	16.7	33	12.5	33	10	20
Ice added to the MCG	0	0	14.2	28.03	10	28.03	7.5	15
Ice added to the batter	23.03	33.03	16.33	0	20.53	0	23.03	13.03
Ice (I total)	23.03	33.03	30.53	28.03	30.53	28.03	30.53	28.03

FC – control formulation. MCG: treatments with fat reduction and lyophilized chia mucilage rehydrated in aqueous solution 24 h before processing at concentrations of 15% (MCG15), 20% (MCG 20) and 25% (MCG25). The following ingredients and/or additives were also used (%) in each treatment: sodium erythorbate, 0.05; sodium nitrite, 0.015; sodium tripolyphosphate, 0.25 and sodium chloride, 2.00.

2.5 Evaluation of meat model systems containing chia mucilage gels (MCG)

2.5.1 Batter analysis before cooking

2.5.1.1 Emulsion stability

Emulsion stability was determined according to Hugues, Cofrades & Troy (1997), with some modification. Approximately 25 g raw meat batter was placed in a plastic tube, centrifuged for 1 min (3600 rpm), heated in a water bath at 70 ºC for 30 minutes, then centrifuged again for 3 min (3600 rpm). Supernatant liquid removed from the samples was

measured. After that, the released liquid was oven dried at 100°C (14 h) and weighed to determine the amount of fat released. Five replicates of each treatment were performed.

2.5.1.2 Penetration force of meat emulsions

Penetration tests were performed at room temperature (25 ºC) using a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) with a load cell of 25 kg coupled with Texture Expert V1.19 software (Stable Micro Systems). Penetration test protocol was obtained from Felisberto, Galvão, Picone, Lopes & Pollonio (2015). Samples were stored at 4°C for 24 h in a cubic container (7 cm side length) for stabilization. The force required to penetrate 2.5 cm of batter was measured at 2 mm/s using a 60° conical probe. Five measurements per sample were obtained.

2.5.2 Meat model system analysis

2.5.2.1 Chemical composition, pH, and water activity

Moisture, protein, and ash content was determined according to AOAC (2005) procedures. Fat content was evaluated according to Bligh & Dyer (1959). Meat model system pH was evaluated using an MA 130 Mettler pH meter with a penetration probe at different places in the sample. Water activity (aw) was measured by an Aqualab water activity meter (Decagon, Pullman, USA). Each treatment was performed in triplicate.

2.5.2.2 Water holding capacity (WHC)

This method was developed in this study to assess the WHC of meat products sliced. Samples were sliced and placed in square plates of expanded polystyrene (12 cm per side) with 3 overlapping slices with filter paper (diameter 11 cm) above and below the sample then vacuum packed. The 3 sample slices (PiS), filter papers (Pifp), and the plastic bag (Pipb) were weighed separately. Samples were then kept at 5°C for two days to simulate commercial storage. After this period, the entire system was then weighed again. Filter papers were then oven dried at 105°C for 2 hours. The volume of liquid released from the slices and the fat percentage were calculated as follows:

Liquid exudation of slices (Les) % = [(weight of the filter paper with the liquid released – Pifp) + (weight of the plastic bag with the liquid released – Pipb)]*100/PiS

Fat exudation of slices (Fes) % = 100*[(Pifp + lipids after drying in an oven) – (Pifp) $\cdot PiS^{-1}$]

All determinations were done in triplicate.

2.5.2.3 Texture profile analysis (TPA)

Texture profile analysis (TPA) was carried out using a TA-xT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) with a load cell of 25 kg. Thirty cylinders per treatment were used. Samples (2 cm thick and 2 cm in diameter) were axially compressed into two consecutive cycles of 30% compression with a 35-mm diameter probe at a constant speed of 1 mm/s. Data were analyzed for hardness (N), springiness (dimensionless), cohesiveness (dimensionless), and chewiness (N).

2.5.2.4 Color measurement

Color was measured in a CM-5 spectrophotometer (Konica Minolta, Tokyo, Japan) using D65 illuminant, 10° observer angle, SCE mode (regarding sample brightness), and CIELab color system to determine parameters L*, a*, and b* as indicators of lightness, redness, and yellowness, respectively. Three readings of each sample were performed at 25 °C. Euclidean distances ($\Delta E= [L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2]^{0.5}$) were calculated (Park, 1994) for comparison between the treatments and control formulation.

2.6 Statistical analysis

Results are expressed as mean ± standard deviation (S.D.) and experiments were conducted in triplicate. Results for all treatments were analyzed by an analysis of variance using a completely randomized design and the general linear model procedure of the Statsoft Inc version 7 software (TIBCO Software Inc., California, USA), considering the treatments as fixed effects and the experiment replication as a random term (n = 3). Tukey's test at 5% significance level ($P \leq 0.05$) was used to determine significant differences between treatments.

3. Results and discussion

3.1 Chemical composition and fatty acid profile of chia mucilage

Table 2 shows the composition and fatty acid profile of lyophilized chia mucilage (MC). Lipid and protein content (26.46% and 11.95%, respectively) may be influenced by the MC extraction process, since the seeds were subjected to intense pressures to separate the mucilage, possibly causing addition lipids and proteins in the mucilage. Felisberto et al. (2015) found similar results to our study for MC protein and dietary fiber content, demonstrating that the extraction method may have a direct influence on the composition obtained, since the authors used a similar extraction process except using a different pulper. Orifici, Capitani, Tomás & Nolasco, (2018) and Capitani, Ixtaina, Nolasco & Tomás, (2013) found higher values of ash (10.3% and 8.4%, respectively) and lower lipid values (9.5% and 3.1%, respectively) than this study, which likely arise from inherent differences in the extraction process and differences in the chia variety and environmental factors (Menga et al., 2017).

The MC composition identified in this study is advantageous for use in meat products, as MC is intended to replace pork back fat, which traditionally elevates saturated fatty acid levels. The fatty acid profile (Table 2) of MC shows significant levels of linolenic acid (56.16%) and linoleic acid (20.64%), which may contribute to a better fatty acid profile of the meat product. Our fatty acid profiles agree with those reported by Ding et al., (2018) and Falco, Amato & Lanzotti, (2017).

Table 2

Proximate composition and fatty acid profile of lyophilized chia mucilage.

Proximate composition (%)	Moisture	Protein	Fat	Ash	Soluble dietary fiber	Insoluble dietary fiber	Total dietary fiber	Digestible carbohydrates
	3.64 ± 0.35	11.95 ± 0.55	26.46 ± 1.30	5.60 ± 0.30	9.91± 0.13	28.39 ± 0.10	38.3 ± 0.24	14.05

Fatty acid (%)	*Myristic C14:0*	*Palmitic C16:0*	*Stearic C18:0*	*Arachidic C20:0*	*Others SFA's*	*Palmitoleic C16:1*	*Margaroleic C17:1*	*Oleic C18:1*	*Eicosenoic C20:1*	*Linoleic C18:2 (n-6)*	*Linolenic C18:3 (n-3)*
	0.1	8.94	4.06	0.49	0.43	0.1	0.04	8.22	0.18	20.64	56.16
	Σ SFA	13.92	*n-6/n-3*	0.37		*Σ MUFA*	8.54			Σ PUFA	76.8
	Atherogenic index	0.11				*Thrombogenic index*	0.14				

* Values represent the average ± standard deviation (*n* =3).

3.2 Rheological properties

Frequency sweeps were performed to investigate time-dependent deformation of MCGs. A stress value within the LVR was chosen and the frequency was increased from 0.1 to 10 Hz while measuring changes in G' and G". Figure 1 shows the changes in storage modulus (G') and loss modulus (G") as a function of frequency (Hz) at 5 °C for 15%, 20%, and 25% w/v MCG. Rheological behavior in mucilage was viscoelastic with a dominant storage modulus (G' > G") forming a structure type gel, where the magnitudes of G′ and G″ slightly increased with raising frequency and had limited frequency dependency. The values of the elastic and viscous moduli were higher at increased MCG concentrations, with the highest elastic and viscous modulus obtained with 25% MC. Mucilage concentration thus influences rheological properties, which has also been reported by other authors (García-Salcedo et al., 2018; Capitani et al., 2015), at higher mucilage concentrations caused higher elastic moduli.

One method to evaluate viscoelastic properties of samples is to measure the tangent of the phase angle (tan δ), which is the ratio of the loss modulus G" to the storage modulus G' and is directly related to the energy lost versus energy stored per cycle. Oscillatory rheological measurements allowed classification of samples as strong gels (G"/G'$\leq$0.1), weak gels (0.1 < G"/G' < 1) and viscous sols (G"/G'$\geq$ 1) as G' and G" indicate solid-like and liquid-like behavior, respectively. For gels, the elastic component (G') dominates over the viscous component (G") at small applied oscillation stresses (Steffe, 1996; Tavernier, Doan, Walle, Danthine, Rimaux & Dewettinck, 2017). For all the concentrations of MCG, tan δ slightly increase as the frequency increased (Fig. 1), with values of tan δ ranging from 0.37 and 0.40, which suggests weak elastic gel-like behavior in the frequency range studied for MCGs (15%, 20% and 25%). This type of behavior has also been reported by other authors for *Plantago lanceolata* seed mucilage (Hesarinejad, Jokandan, Mohammadifar, Koocheki, Razavi, Ale, et al., 2018), basil seed mucilage (Samateh, Pottackal, Manafirasi, Vidyasagar, Maldarelli & John, 2018), and *Pereskia aculeata Miller* mucilage (Amaral, Junqueira, Tavares, Oliveira, Prado & Resende, 2019).

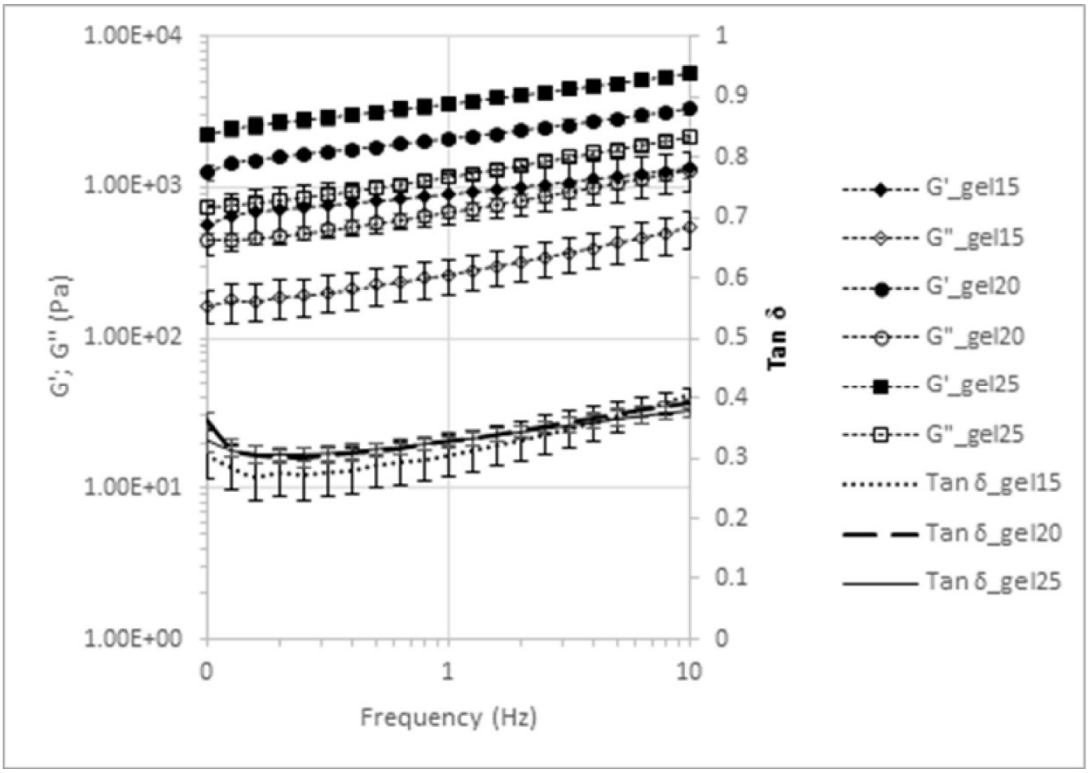

Fig. 1. Frequency sweep evaluating different chia mucilage concentrations on storage (*G'*), loss (*G''*) moduli and tan δ at constant temperature of 5 °C. Solid symbols are corresponding to *G'* and open symbols *G''*. Gel15, gel20 and gel25 – lyophilized chia mucilage rehydrated in aqueous solution 24 h before analysis at respective concentrations.

The effects of heating to 72°C on G' on G'' for different concentrations of MCG are shown in Fig. 2. MCG gels had a higher storage modulus compared to the loss modulus over the whole temperature range evaluated, although both G', and G'' slightly decreased with increased temperature, which may be related to the greater fluidity of the gels at higher temperatures. However, all concentrations of MCG are temperature stable with few variations in mechanical properties as reflected by the G', and G" values. Moreover, these parameters are not much lower than those obtained at 5 °C during the frequency sweep demonstrated in Fig. 1. The frequency sweep performed at 72°C (Fig. 2b, 2d, and 2f) shows that mehanical behavior was essentially frequency independent, with plots of G' and G" vs frequency being almost linear, demonstrating that these gels have excellent tolerance to variations in deformation rate. For weak gels, according to Martínez-Ruvalcaba, Chornet and Rodrigue (2007), both moduli show slight frequency dependencies and G' exceeds G" at all frequencies typically examined.

This result is consistent with previous observations of the effects of temperature on chia seed polysaccharides, as raising the temperature to 80°C did not alter the weak gel properties of the polysaccharide dispersions (Goh, Matia-Merino, Chiang, Quek, Soh & Lentle,

(2016)). Although the concentrations of dispersions evaluated in this study are much higher than most studies with MC in order to test the use of MC in meat products, the rheological characteristics remain similar.

Fig 3a, 3c, and 3e show the temperature sweeps for different MCG concentrations, simulating the temperature changes that occur during the cooking process of a typical emulsified meat product. Fig 3b, 3d, and 3f show frequency sweeps at 5°C after temperature variation and indicate that the viscoelastic behavior of the MC dispersions is preserved even after temperature changes, with values of G' and G" remaining fairly consistent (Fig. 1) before and after temperature oscillations. Such results are essential for use of MC as an ingredient in food processing since it provides information about MC stability during temperatures that a meat product is subjected during its processing.

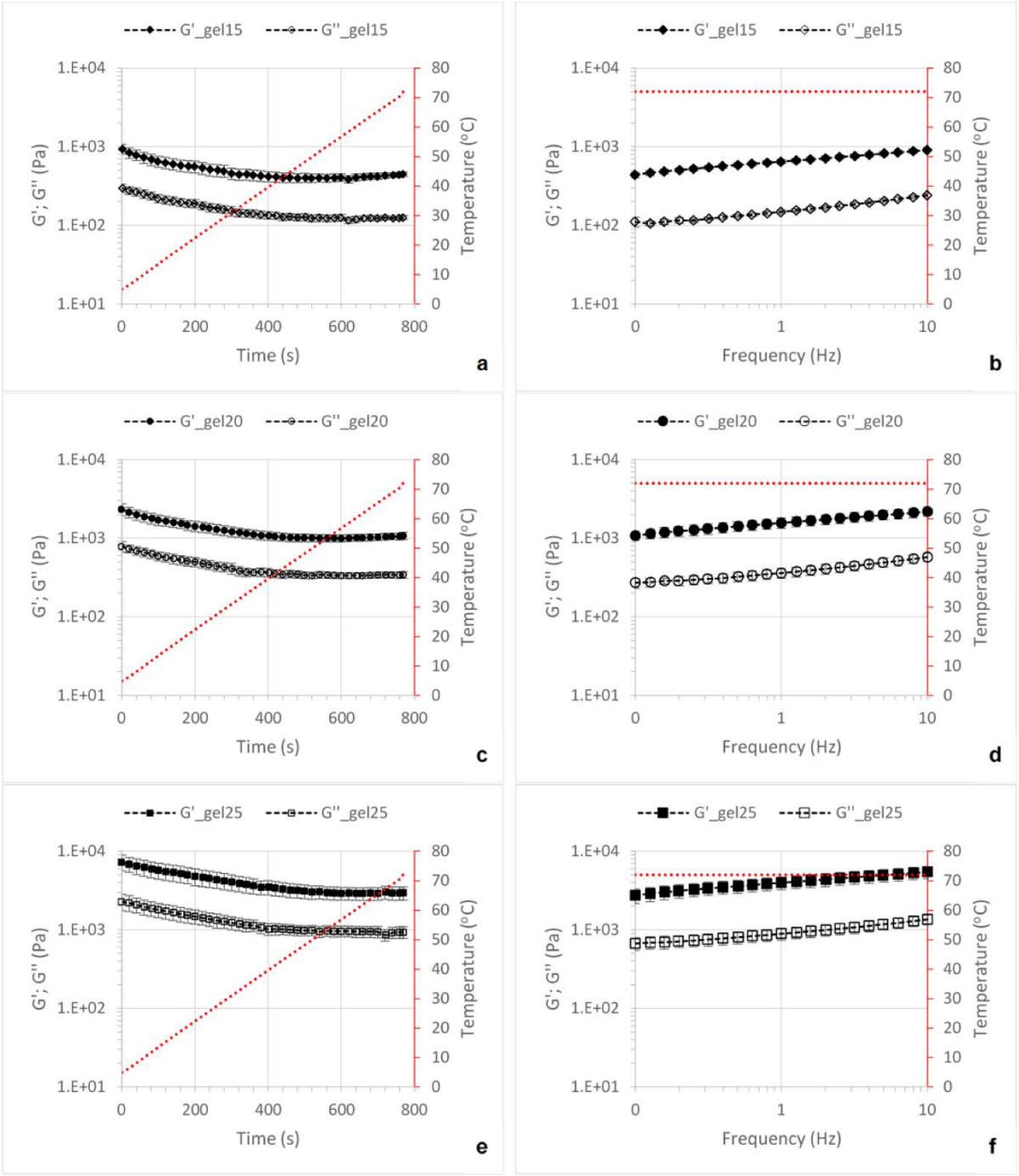

Fig. 2. Temperature sweeps (heating stage) are shown for systems prepared with different concentration of chia mucilage (a, c and e), and frequency sweep at 72 °C for corresponding suspensions after increase of temperature (b, d and f). Solid symbols are corresponding to *G′* and open symbols *G″*. Gel15, gel20 and gel25 - lyophilized chia mucilage rehydrated in aqueous solution 24 h before analysis at respective concentrations.

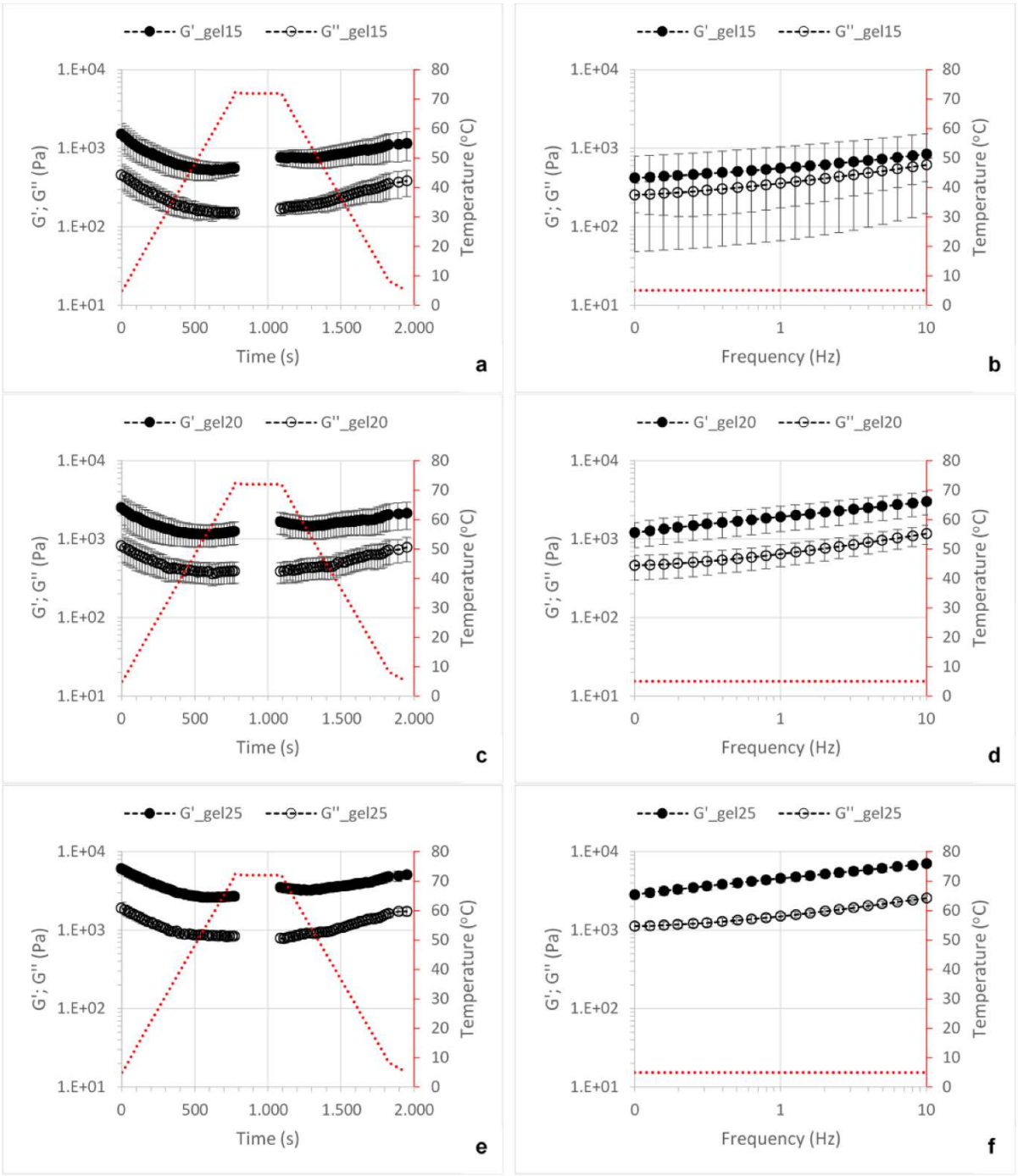

Fig. 3. Temperature sweeps (heating and cooling stages) are shown for systems prepared with different concentration of chia mucilage (a, c and e), and frequency sweep at 5 °C for corresponding suspensions after temperature changes (b, d and f). Gel15, gel20 and gel25 - lyophilized chia mucilage rehydrated in aqueous solution 24 h before analysis at respective concentrations.

3.3 ATR-FTIR analysis

The ATR-IR spectrum of extracted mucilage is presented in Fig. 4. The band between 3500 and 3100 cm^{-1}, which in mucilage spectra is centered at 3286 cm^{-1}, represents the hydroxyl -OH stretching originating from carbohydrate structures (Cerqueira, Souza, Simões, Teixeira, Domingues, Coimbra, et al., 2011). Other authors have identified this spectral band in purified gum from chia seeds (Timilsena et al., 2016). Another band was observed at 3012 cm^{-1} and attributed to the stretching vibration of a =C-H group. Two strong bands also appear at 2924 and 2854 cm^{-1} (Fig. 4) and are identified as the asymmetric and symmetric stretching vibrations, respectively, of acyl CH_2 groups (Guillen & Cabo, 1997, Herrero et al., 2006; Herrero et al., 2011). Similarly, such infrared bands in this spectral region have been described for chia flour and mucilage (García-Salcedo et al., 2018).

The infrared bands in the 2700-3000 cm^{-1} region (Fig. 4) are characteristic of lipid and protein functional groups (Surewicz & Mantsch, 1996; Herrero et al., 2006) presented in the extracted mucilage (Table 2). A strong band was located at 1743 cm^{-1} and likely represents stretching modes of the carbonyl groups C=O. The amide I band in the 1700 to 1600 cm^{-1} region (Fig. 4) is the most characteristic band for proteins (Surewicz & Mantsch, 1996). The broader band between 1198 and 938 cm^{-1} (Fig. 4) likely results from the stretching vibration of C–O in C–O–H bonds (e.g., glycosidic bonds) and is related to the carbohydrates of mucilage. Notably, the band at 1155 cm^{-1} corresponds to bending vibrational modes of C–O in the pyranose ring, while the band between 1124 and 938 cm^{-1} is characteristic of C-O-H bending (Cerqueira et al. 2011). In this spectral region, the band centered at 1036 cm^{-1} can be assigned to C-O-C stretching of glycosidic bonds and C-O-H bending, considered characteristic of polysaccharide compounds (Cerqueira et al., 2011). In purified gum from chia seed, similar infrared bands were identified in this spectral region (Timilsena et al., 2016).

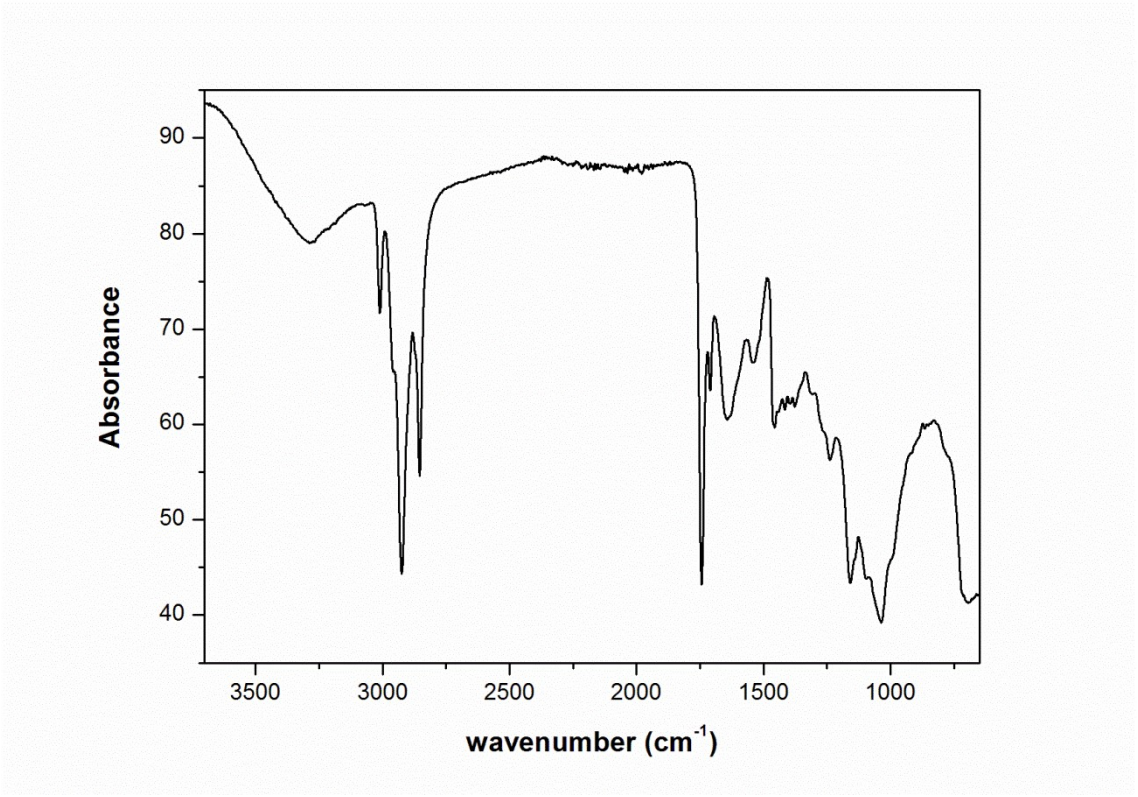

Fig. 4. Typical spectrum of extracted chia mucilage (powder) in the 3700- 650 cm^{-1} region.

3.4 Emulsion stability of the batters prior to thermal processing

Stability of batters of the different treatments wasn't affected by fat reduction or the addition of chia mucilage gels (MCGs) (Table 3). Both liquid exudation and fat exudation values of meat batters containing MCG did not differ (P> 0.05) from the control (FC1), independent of the concentration of gel. The higher stability of the samples with added MCG may be attributed to the water holding capacity of the MC. According to Muñoz et al. (2012), MC has great hydration capacity as a 100 mg sample of mucilage was able to absorb 2.7 g of water, or 27 times its weight. Additionally, fat reduction and the addition of the MCGs increased the moisture/protein ratio, as higher amounts of ice were added to the formulations, although there was no significant liquid release in the reformulated samples, which reinforces and confirms the increased stability observed for products containing MCGs.

Table 3. Chemical composition and physicochemical properties of meat model systems with chia mucilage gels (MCGs) in different concentrations.

	FC1	FC2	MCG15-2.5%	MCG15-5%	MCG20-2.5%	MCG20-5%	MCG25-2.5%	MCG25-5%
Chemical composition (%)								
Moisture	67.89±0.10^{g}	76.03±0.03^{a}	73.65±0.02^{b}	71.20±0.01^{d}	72.95±0.02^{c}	70.78±0.03^{f}	73.63±0.03^{b}	70.98±0.01^{e}
Protein	13.60±0.14^{a}	12.86±0.42^{b}	13.13±0.27^{a,b}	13.19±0.11^{a,b}	12.96±0.42^{b}	13.01±0.28^{b}	12.99±0.36^{b}	13.32±0.15^{a,b}
Fat	16.52±0.34^{a}	8.99±0.09^{d}	9.59±0.04^{c}	10.49±0.16^{b}	9.25±0.14^{c}	10.73±0.06^{b}	9.55±0.47^{c}	10.55±0.14^{b}
Ash	2.94±0.02^{b}	2.83±0.04^{c}	2.96±0.01^{b}	3.08±0.02^{a}	2.99±0.01^{b}	3.11±0.03^{a}	2.61±0.02^{d}	2.81±0.01^{c}
Physico-chemical properties								
pH	6.69±0.04^{a}	6.71±0.03^{a}	6.47±0.02^{c}	6.41±0.02^{d}	6.36±0.03^{d}	6.37±0.02^{d}	6.46±0.04^{c}	6.57±0.04^{b}
Aw	0.972±0.00^{c,d,e}	0.975±0.00^{a,b,c}	0.974±0.00$^{a,\ b,c,d}$	0.976±0.00^{a,b}	0.970±0.00^{e}	0.971±0.00^{d,e}	0.972±0.00^{b,c,d,e}	0.976±0.00^{a}
Emulsion stability (%)								
Liquid exudation	0.47±0.26^{b}	1.36±0.49^{a}	0.47±0.21^{b}	0.26±0.10^{b}	0.84±0.15^{a,b}	0.71±0.19^{a,b}	0.61±0.14^{b}	0.47±0.27^{b}
Fat exudation	0.04±0.02^{b}	0.11±0.02^{a}	0.04±0.01^{b}	0.02±0.01^{b}	0.05±0.00^{b}	0.05±0.01^{b}	0.04±0.01^{b}	0.03±0.01^{b}
Liquid exudation of slices (Les) after 2 days (5ºC) (%)	20.33±1.14^{a}	19.09±0.52^{a,b}	19.05±0.17^{a,b}	18.28±0.93^{b}	18.14±0.33^{b}	19.35±0.11^{a,b}	19.02±0.65^{a,b}	17.95±0.23^{b}
Fat exudation of slices (Fes) after 2 days (5ºC) (%)	1.12±0.04^{a}	0.71±0.14^{b}	0.96±0.14^{a,b}	1.25±0.20^{a}	0.89±0.11^{a,b}	1.24±0.10^{a}	0.91±0.10^{a,b}	1.02±0.22^{a,b}

* Values represent the average ± standard deviation. ^{a,b,c,d,e,f,g} Means in the same row with the same letters did not differ significantly at $P < 0.05$. [1]For treatments denominations, see Table 1.

3.5 Chemical composition, physicochemical, and technological properties of meat model systems with MCG

Chemical composition of meat model systems showed some significant differences between treatments (Table 3). The moisture content of the control formulation (FC1) was significantly lower ($P < 0.05$) than any other treatments. FC2, on the other hand, had reduced fat and, without the addition of MCG, showed the highest value for this parameter ($P<0.05$). These results are consistent with the reformulation strategy adopted, since the ice was added to balance the formulations with reduced fat and was used in the MC dispersions, in agreement with previous studies (Paglarini et al., 2019; Pintado, Herrero, Ruiz-Capillas, Triki, Carmona & Jimenez-Colmenero, 2015). Samples with MCG had moisture values ranging between 70.78% (MCG20-5%) and 73.65% (MCG15-2.5%), which were significantly higher ($P < 0.05$) than those of FC1. Protein content of fat-reduced formulations were slightly lower than FC1, due to the higher amount of residual protein found in pork back fat. The final fat content in all treatments with MCG was around 10% and was slightly higher in treatments with 5% MCG due to the amount of lipids present in MC (Table 2), which is lower than the control formulation (16.52%). Thus, these meat products containing MCGs can be claimed reduced fat, according to European Regulation on nutrition claims (EC, 2006), since they have a fat reduction of approximately 35% when compared to control treatment (FC1). The ash contents were increased ($P < 0.05$) in formulations with 5% MCG, likely due to the high mineral content of MC (see Table 2).

Regarding physicochemical properties, pH values in meat model systems ranged between 6.71 (FC2) and 6.36 (MCG20-2.5%) (Table 3). Lower pH values in MCG treatments was expected as MC dispersions had pH values around 6.0 (data not shown). For aw, except for MCG15-5% and MCG25-5%, no significant differences ($P<0.05$) were observed between formulations with MCG and the control (FC1).

The replacement of pork fat by MCG also influenced the water retention capacity of sliced samples based on measurements of liquid and fat exuded from slices, denoted Les and Fes, of meat model systems (Table 3). Lower values of Les were obtained for treatments MCG15-5%, MCG20-2.5%, and MCG25-5%, which differed significantly ($P<0.05$) from that of FC1. The Les parameter is an attempt to quantify a frequent problem that occurs at the commercialization point of sliced sausages, as the lower exudation of liquid in sliced meat products can be considered a mark of higher by consumers. Due to its thickening and CRA properties (Muñoz et al., 2012; Capitani et al., 2015), MC appears an effective agent for

retaining water in meat model systems, as long as efficient incorporation (MC) is performed at the moment of processing.

3.5.1 Textural parameters

Texture profile showed that reducing fat content and adding MCG affected the texture properties of meat model systems (Table 4). The lowest ($P<0.05$) hardness and chewiness values were found in FC2, which has reduced fat and no MCG. Fat plays an essential role in the texture and water-binding capacity of meat products, stabilize the solubilized protein gel network in emulsified meat products (Jimenez-Colmenero, 1996). Moreover, in FC2, when fat content was reduced, the amount of water was increased, resulting in lower binding properties, with a less firm texture, in line with the findings of Álvarez & Barbut, (2013).

For reformulated products, the impact of MCG addition on the hardness and chewiness were similar, with higher values of both parameters in MCG20-2.5%, MCG20-5%, MCG25-2.5% and MCG25-5% formulations compared to controls (FC1 and FC2). This observation was expected, as chewiness is a secondary parameter that typically reflects hardness values (Selgas, Cáceres, & García, 2005). These textures can be attributed to the properties and composition of MC, which has high fiber, thickening properties, and high-water retention capacity. Moreover, fiber addition has been shown to increase the hardness and chewiness of meat products (Han & Bertram, 2017; Pintado, Herrero, Jiménez-Colmenero & Ruiz-Capillas, 2016).

Concerning to the different concentrations of MC dispersions (15%, 20%, and 25%) evaluated in this study, it is possible to observe the similarity between the parameters hardness, chewiness and penetration force for the control (FC1), MCG15-2.5% and MCG15-5 %. These concentrations produced more fluid gels which, when incorporated into the meat batter, provided texture properties more similar to the control concerning for these parameters, independent of the final concentration of MC in the product (2.5% or 5%).

The final concentration of MC did impact elasticity and cohesiveness of meat model systems. All formulations with 5% MC, regardless of MCG concentration, showed decreased elasticity and cohesiveness values compared to FC1 and FC2 controls ($P<0.05$). One potential explanation is that dietary fiber from MC interferes aggregation of myosin globular heads, which is the first step of the protein gelation process, occurring at higher temperatures. The gelation of myosin provides greater elasticity to meat systems mainly due to the hydrophobic and disulfide-sulfhydryl interactions between proteins (Savadkoohi, Shamsi, Hoogenkamp,

Javadi & Farahnaky, 2013; Ferris, Sandoval, Barreiro, Sánchez & Müller, 2009). MC may interfere with these mechanisms, resulting in less elastic and cohesive products. Pintado et al. (2016) found similar results, as Frankfurters made with chia flour showed lower cohesiveness and springiness compared to control.

3.5.2 Color parameters

Color is one of the most relevant quality attributes of a meat product because it has a direct impact on consumer acceptance. Table 4 shows the values for L*, a*, and b*. All the color parameters evaluated differed ($P<0.05$) among the different treatments with MCG. Meat model systems containing MCG had reduced lightness (L*) ($P <0.05$), when the concentration of this component increased. Meat model systems with MCG also had lower values of a* and higher values of b*, which differed significantly ($P <0.05$) from the control samples (FC1 and FC2). These results are directly related to the brownish color MC (instrumental color values were 52.19 ± 0.79 for L*; 5.26 ± 0.14 for a*, and 20.01 ± 0.52 for b*, data not shown), illustrated in Fig 5. Similar results were reported by Pintado et al. (2016), who observed a reduction in L* and a* and an increased in b* in Frankfurters with the addition 10% of chia flour. Such effects on Frankfurter color due to the chia coproduct (3%) have also reported by Fernández-López, Lucas-González, Viuda-Martos, Sayas-Barberá, Navarro, Haros, et al., (2019).

Table 4

Texture and color parameters of meat model systems with chia mucilage gels (MCGs) in different concentrations.

	FC1[1]	FC2	MCG15-2.5%	MCG15-5%	MCG20-2.5%	MCG20-5%	MCG25-2.5%	MCG25-5%
Textural parameters								
Hardness (N)	7.67±0.72^{e}	5.59±0.62^{f}	8.97±0.80^{c,d}	8.31±0.95^{d,e}	10.42±0.79^{a}	10.63±0.98^{a}	9.57±0.85^{b,c}	10.01±0.94^{a,b}
Springiness	0.921±0.02^{a}	0.922±0.01^{a}	0.918±0.01^{a}	0.887±0.02^{b}	0.908±0.02^{a}	0.893±0.02^{b}	0.909±0.02^{a}	0.883±0.02^{b}
Cohesiveness	0.82±0.01^{a}	0.81±0.02^{a}	0.796±0.01^{b}	0.756±0.02^{e}	0.772±0.01^{d}	0.762±0.02^{d,e}	0.785±0.01^{c}	0.757±0.01^{e}
Chewiness (N)	5.78±0.52^{c}	4.20±0.47^{d}	6.55±0.58^{b}	5.58±0.70^{c}	7.31±0.52^{a}	7.23±0.80^{a}	6.83±0.62^{a,b}	6.70±0.66^{b}
Penetration force (N)	3.65±0.08^{b,c}	2.90±0.26^{d}	4.00±0.14^{a,b}	3.85±0.13^{a,b,c}	4.21±0.22^{a}	4.28±0.61^{a}	3.49±0.17^{b,c}	3.42±0.10^{c,d}
Color parameters								
L*	67.39±0.54^{a}	65.64±0.37^{b}	63.59±0.38^{d}	60.33±0.51^{g}	62.84±0.41^{e}	61.33±0.28^{f}	64.66±0.51^{c}	61.7±0.15^{f}
a*	14.54±0.36^{b}	15.19±0.22^{a}	10.58±0.13^{c}	9.55±0.35^{d}	10.91±0.09^{c}	9.25±0.19^{d}	10.58±0.11^{c}	9.17±0.38^{d}
b*	6.51±0.18^{e}	5.34±0.11^{f}	11.28±0.22^{c}	14.45±0.35^{a}	10.92±0.15^{d}	14.10±0.35^{b}	11.10±0.12^{c,d}	14.23±0.23^{a,b}
ΔE	—	2.22±0.27^{e}	7.28±0.23^{c}	11.75±0.42^{a}	7.31±0.28^{c}	11.07±0.17^{b}	6.67±0.19^{d}	10.99±0.23^{b}

*Values represent the average ± standard deviation. ^{a,b,c,d,e,f,g} Means in the same row with the same letters did not differ significantly at $P > 0.05$. [1] For treatments denominations, see Table 1.

Fig. 5. Appearance of chia seeds, chia mucilage (MC) and chia mucilage gel (MCG) in different concentrations

4. Conclusions

In this study, we showed that MC was a viable substitute for fat in meat products. The mucilage concentration in MCGs influences rheological properties, and the values of the elastic and viscous moduli were higher with increased MCG concentrations. The values of tan δ suggest that MC can form weak elastic gels for the frequency range tested. The rheological behavior of the MCGs demonstrated that their mechanical properties are resistant to thermal stress.

Meat model systems with various concentrations of MCGs behaved similarly, with the level of MC (2.5% or 5%) in the products having a more substantial impact on meat model system properties. There were significant improvements in the stability of meat emulsions and decreased exudation by sliced products, which are relevant quality parameters for meat products technology and commercialization. However, MC concentration in the final product had a significant influence on texture and color. Concentrations of 5% MC increased hardness and decreased elasticity and cohesiveness of products, providing evidence that they interfere in some way with the molecular linkage of the emulsified meat matrix. Additionally, color was negatively impacted and could be considered a technological barrier to be overcome. Despite this, chia mucilage represents a promising fat substitute in meat products, although further studies on the microstructure, water mobility, and interactions between the MC and the meat matrix are still necessary.

Declarations of interest

The authors declare that they have no conflict of interest.

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CAPÍTULO 3

REDUCING PHOSPHATES IN EMULSIFIED MEAT PRODUCTS WITH POWDER OR GEL OF CHIA (*SALVIA HISPANICA* L.) MUCILAGE: A CLEAN LABEL TECHNOLOGICAL STRATEGY.

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Reducing phosphates in emulsified meat products with powder or gel of chia (*Salvia hispanica* L.) mucilage: a clean label technological strategy

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Abstract

The objective of this study was to assess the potential of chia mucilage (MC) to serve as a substitute for phosphates in low-fat Bologna sausages through the evaluation of its functional properties, such as water-holding capacity, gel and emulsion formation ability, texture, and physicochemical and sensory properties. A total of six treatment samples with 50% fat reduction and the addition of 2% and 4% chia mucilage as a powder (MCP) and gel (MCG) were prepared; four of these treatments were produced without the addition of phosphates (F1- 2% MCP; F2- 2% MCG; F3-4% MCP; F4- 4% MCG) and two were prepared with the addition of 0.25% phosphate (F5 - 2% MCG and F6 - 4% MCG). A control treatment (FC) with 20% fat and 0.5% phosphate was also prepared. The L* and a* values were lower ($P<0.05$) in case of all treatments, compared to FC. There were no differences ($P>0.05$) between the elasticity values of the samples FC, F2, and F5. Treatments F4 and F6 showed a spongy and less cohesive protein structure. F3, F4, and F6 (all containing 4% MC) exhibited lower relaxation times and more restricted water mobility. Treatments containing 2% MCG did not differ ($P>0.05$) from FC with regards to the overall acceptance. Thus, utilizing chia mucilage is a viable clean-label strategy to substitute the use of phosphates in low-fat Bologna sausages.

Keywords: low-fat Bologna sausages; chia mucilage; *Salvia hispanica* L.; reduction of phosphates; water mobility; clean label.

1. Introduction

Currently, there is a shift in the eating patterns in our society, with consumers being increasingly aware of the relationship between food and health. In this context, meat products are often perceived as unhealthy; however, many of the negative connotations of these products can be overcome by reducing the levels of harmful constituents, such as saturated fats, salt, phosphates, and nitrites (Grasso, Brunton, Lyng, Lalor, & Monahan, 2014). However, scientific studies have indicated that reducing the levels of these components can lead to several technological limitations in meat products. Therefore, there is an increasing trend of introducing "healthier" functional ingredients, such as fibers, non-meat proteins, and oils with more favorable fatty acid profiles, which can, to some extent, minimize the potential defects (with regards to texture, color, stability, and so on) caused by the reduction of the levels of these

compounds (Choe, Lee, Jo, Jo, Song, & Jung, 2018; Hjelm, Mielby, Gregersen, Eggers, & Bertram, 2019; Câmara & Pollonio, 2015).

Phosphates are synthetic additives widely used in meat products; they are considered fundamental components in meat-based products due to their functional properties, such as their water-holding capacity, ability to displace the isoelectric point of myofibrillar proteins, thereby increasing their solubility and extraction potential, and potent antioxidant activity (Dušek, Kvasnička, Lukášková, & Krátká, 2003). Therefore, phosphates are used to reduce cooking losses, retard lipid oxidation, maintain the color and texture properties, and protect the foodstuffs against microbial growth (Aberle, Forrest, Gerrard, & Mills, 2001). As a result of their mechanism of action, phosphates are important additives in the processing of meat products, and for this reason, phosphate reduction is considered a challenging aspect.

It is estimated that approximately 50% of the daily intake of phosphorus (P) comes from food additives containing P, such as phosphates, which are widely used in the meat industry and rarely quantified on the labels (Winger, Uribarri, & Lloyd, 2012). For example, this is not legally required in Brazil. It is well known that hyperphosphatemia, which is defined as the excessive dietary intake of phosphates, can harm the health of a specific group of consumers, particularly, people with kidney disease (Benini, Alessandro, Gianfaldoni, & Cupistini, 2011). Chronic kidney disease results in an inappropriate excretion of phosphorus (P), which is considered very serious, as it is directly related to mortality and morbidity (Winger et al., 2012). A high serum phosphate concentration is also an independent predisposing factor associated with coronary atherosclerosis in the general population (Ritz, Hahn, Ketteler, Kuhlmann, & Mann, 2012); therefore, strategic meat product reformulations to reduce phosphate levels are promoted.

Chia mucilage is a water-soluble anionic heteropolysaccharide that is exuded when chia seeds are soaked in water; it is composed of β-D-xylose, α-D-glucose, and 4-O-methyl-α-D-glucuronic acid in a ratio of 2:1:1, respectively (Lin, Daniel, & Whistler, 1994). Chia mucilage has been described as a novel source of polysaccharide gels with exceptional water-holding properties; these gels can have important applications in the food industry as thickening agents and for the process of gel formation and the stabilization of emulsions (Capitani, Nolasco, & Tomas, 2016; Muñoz, Cobos, Diaz, & Aguilera, 2012; Capitani et al., 2015). In addition, health benefits associated with its ingestion, such as the slowing down of the digestion process, increased perception of satiety, prolonged glucose release, and reduced intestinal absorption of fatty acids and cholesterol, have been reported (Hentry, Mittleman, & McCrohan, 1990). Considering both its technological properties of water-holding capacity and gel

formation ability, and health benefits, the hypothesis that chia mucilage could substitute phosphates and fats in meat products has emerged as an alternative for a strategic meat product reformulation with the objective of producing healthier products. In addition, replacing a synthetic additive with a natural ingredient contributes to the development of clean-label strategies. Therefore, the objective of this study was to assess the potential use of chia mucilage (in the form of a powder and gel) as a substitute for phosphates in low-fat Bologna sausages through the evaluation of its functional properties, such as water-holding capacity, gel and emulsion formation, texture, and physicochemical and sensory properties.

2. Materials and methods

2.1 Materials

Pork (*longissimus thoracis et lumborum*) (69.3% moisture, 8.6% lipids, 20.4% protein, and 1.7% ash), beef (*quadriceps femoris)* (73.21% moisture, 3.81% lipids, 21.63% protein, and 1.35% ash) and pork back fat (11.1% moisture, 81.2% lipids, 7.05% protein, and 0,65% ash) were obtained from a local market in Campinas (Brazil). After cleaning to remove apparent fat and aponeuroses, meat and pork fat were ground in a 5.0 mm disks, packed in vacuum and then frozen (-18 ºC) until further use. Chia seeds (*Salvia hispanica* L.) were provided by Cereal Prime (São Paulo, Brazil). Sodium tripolyphosphate, sodium erythorbate, and sodium nitrite were kindly donated by Kerry do Brasil (Campinas, Brazil). Nile red dye and fluorescein isothiocyanate (FITC) were purchased from Sigma (Sigma–Aldrich Corporation St. Louis, USA).

2.2 Extraction of chia (Salvia hispanica L.) mucilage

Chia mucilage (MC) extraction was performed according to the method proposed by Coorey et al. (2014) and Felisberto et al. (2015) with some modifications. Briefly, chia seeds were placed in an electric cooker (STM, São Paulo, Brazil) and distilled water was added in 1:25 proportion for 3 h at 60 ºC. The mucilage was separated from the chia seeds using a 35/ CM-876 finisher depulper with a stainless-steel wire mesh of aperture size 0.26 mm (FMC do Brasil Indústria e Comércio Ltda, Araraquara, Brazil). After that, the mucilaginous gel was dried in an LP 820 freeze-drier (São Paulo, Brazil), was ground using a food processor (GM

200, Retsch, Germany) to obtain a fine powder, and packaged in hermetically sealed metalized packaging, protected from moisture.

2.3 Experimental design and Bologna sausages preparation with chia mucilage powder (MCP) and chia mucilage gel (MCG).

Six treatments were elaborated with 50% fat reduction and 2 and 4% MC addition, with two different forms of incorporation in the meat batter: as a powder (MCP) and as a gel (MCG). Of these six treatments, four were produced without the addition of phosphates (F1, F2, F3, and F4) and two were made with 50% reduction of this additive (F5 and F6). Besides one control treatment (FC), with 20% of pork back fat and 0.5% of phosphate, as described in Table 1. The following ingredients were also added in percent (%) in each of the treatments: sodium chloride, 1.5%; sodium nitrite, 0.015%; condiments, 1.34%; and sodium erythorbate, 0.05%. For mucilage incorporation as a gel, the lyophilized mucilage was rehydrated in water, at 25 % of concentration (concentration assessed in preliminary tests), and allowed to stand overnight before processing at a temperature of 4 ºC

To elaborate the Bologna sausages, partially thawed ground meat (5.0 mm disk, -0.5 ºC), sodium chloride, sodium tripolyphosphate (depending on the formulation), and ice were placed in the cutter (model MTK 662, Mado, Dornhan, Germany) for extraction of myofibrillar proteins. Added the other additives slowly, and pork back fat or MCP/MCG was added at the end, followed by comminution until complete homogenization. During comminution, the temperature of the meat batter did not exceed 12 °C. Part of the amount of batter made was stored raw, packed in plastic containers and kept at 4 °C until the emulsion stability analysis (maximum period of 5 h). The other part of the meat batters was stuffed into water-impermeable plastic casings (Spel Embalagens, Brazil) of 85-90 mm in diameter with approximately 0.4 kg of product per package. The Bologna sausages were cooked in an oven (Arprotec®, Brazil), where the temperature was gradually increased until the internal temperature reached 72 ºC. The products were then cooled in ice and stored under refrigeration (4 ºC) until analysis.

Table 1

Description of treatments (%, w/w) of Bologna sausages elaborated with chia mucilage powder (MCP) and chia mucilage in gel (MCG).

Ingredients	Treatments (%)						
	FC	F1	F2	F3	F4	F5	F6
Pork	28	28	28	28	28	28	28
Beef	32	32	32	32	32	32	32
Pork back fat	20	10	10	10	10	10	10
Chia mucilage powder-total (MCP)	0	2	2	4	4	2	4
Chia mucilage in gel (MCG)	0	0	8	0	16	8	16
Ice added to the MCG	*0*	*0*	*6*	*0*	*12*	*6*	*12*
Ice added to the batter	*16.55*	*25.05*	*19.05*	*23.05*	*11.05*	*18.80*	*10.80*
Ice (I total)	16.55	25.05	25.05	23.05	23.05	24.80	22.80
Sodium tripolyphosphate	*0.5*	*0*	*0*	*0*	*0*	*0.25*	*0.25*

FC – control formulation; The following ingredients and/or additives were also used (%) in each treatment: condiments,1.385; sodium erythorbate, 0.05; sodium nitrite, 0.015; and sodium chloride, 1.5.

2.4 Proximate composition, pH and aw

Proximate composition including moisture, ash and protein content of Bologna sausages was determined according to the methodology described by the Association of Official Analytical Chemists (AOAC, 2005). The fat content was measured following the method of Bligh and Dyer (1959). The determination of phosphates was carried out from the ashes obtained from the samples by spectrophotometric method which is based on the formation of phosphomolybdate with added molybdate followed by its reduction with sodium sulphide in aqueous acidic medium. The readings were made at 420 nm (Instituto Adolfo Lutz, 2005). The pH of samples was evaluated using a MA 130 Mettler pH meter using a penetration probe at different places of the sample. The water activity (aw) was measured by an Aqualab water activity meter (Decagon, Pullman, USA). Three samples of each replication were carried out and the average values were used for statistical analysis.

2.5 Emulsion stability

Emulsion stability was determined in five replicates for each treatment using the methodology proposed by Hughes, Cofrades & Troy (1997), with some modifications. Approximately 25 g raw meat batter of each treatment was placed in plastic tubes, centrifuged for 1 minute (3600 rpm), heated in a water bath at 70 ºC for 30 minutes and centrifuged again for 3 minutes (3600 rpm). The supernatant liquid removed from the samples was measured. After that, the released liquid was oven dried at 100 °C (14 h) and weighed to determine the fat released.

2.6 Color determination

Color of all samples was measured using a CM-5 spectrophotometer (Konica Minolta, Tokyo, Japan), operating with D65 illuminant, 10° observer angle, SCE mode (regarding sample brightness), and CIELab color system for the evaluation of parameters L*, a*, and b*. The color variables were measured at three points on the central part of the cut surface of three slices of the samples at room temperature (25 ºC). L*, a*, and b* values were determined as indicators of lightness, redness, and yellowness, respectively. Whiteness was calculated from the L*, a* and b* values: $\text{Whiteness} = 100 - [\,(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5}$. The Bologna sausages were evaluated 24 h after processing and every 15 days during 60 days of shelf-life.

2.7 Texture profile analysis (TPA)

Texture profile analysis (TPA) was evaluated at room temperature in TA-xT2i texture analyzer (Texture Technologies Corp., Scarsdale, NY) with a load cell of 25 Kg. Thirty cylinders per treatment were used. Samples (2 cm thick and 2 cm diameter) were axially compressed into two consecutive cycles of 30% compression, with a 35 mm diameter probe, at a constant speed of 1 mm/s. Data were analyzed for hardness (N), springiness (dimensionless), cohesiveness (dimensionless), and chewiness (N). The samples were evaluated at 24 h after processing and every 15 days during 60 days of shelf-life.

2.8 Confocal laser scanning microscopy (CLSM)

A Leica TCS SP5II (Leica Microsystems Heidelberg, Germany) equipped with a helium/neon laser was used for the fluorescence excitation (500-530 nm for FITC and 505-586 nm for Nile Red). Nile red (0.02% w/v in methanol) and Fluorescein Isothiocyanate- FITC (0.02% w/v in acetone) was used to stain fat and protein, respectively. Thin slices of Bologna sausages were cut with a scalpel and stained with ten microliters of each dye. The observations were made 10 min after diffusion of the dyes into the sample. Data from representative areas for each sample were taken using a 10X magnification objective, scale bar of 250 μm.

2.9 Low field NMR analysis

NMR relaxation measurements were performed using a MiniSpec mq 20 NNR spectrometer (Bruker BioSpin, Rheinstetten, Germany) operating at a frequency of 20 MHz. The methods followed those of Bertram, Andersen & Andersen (2007) with some modifications. The samples of Bologna sausages with approximately 1.0 g were directly placed in an 8-mm glass tube and inserted in the NMR probe, and waiting about 15 min was to stabilize the temperature thermalized at 39.8 °C. The T_2 measurements were performed with a τ-value (time between 90° pulse and 180° proton pulses of 8.5 and 16.6 μs, respectively) of 160 μs. The spin–spin relaxation time, t_2, was measured using the Carr-Purcell-Meiboom-Gill sequence. The data, performed were obtained in triplicate in for two different batches samples, from in which 20000 echoes were acquired as with 16 scan repetitions with and the repetition time between subsequent scans of was 15 s. Areas and relaxation times of the relaxation populations found were calculated.

2.10 Sensory evaluations

The sensory studies were approved by the Ethics in Research Committee of the University of Campinas, SP, Brazil (CAAE – 6815 1217.8.0000.5404) and all participants signed a free and informed consent form, agreeing voluntarily to participate in the sensory tests. All tests were performed in the Sensory Analysis Laboratory (DTA/UNICAMP) using individual booths with lighting and proper temperatures, ensuring the comfort and privacy of participants. For the sensorial evaluations, the treatments FC, F2, F4, F5, and F6 were selected. The criteria taken into account for the choice of treatments were better stability of the meat emulsion and textural parameters, such as elasticity, of samples with MCG compared to those with MC powder, as will be discussed in the development of this work.

2.10.1 Temporal Dominance of Sensation (TDS)

All the panellists (n = 12) were familiar with sensory evaluation and were habitual consumers of meat products. Three preliminary sessions were conducted. The panellists were introduced to the notion of the temporality of sensations and to the TDS technique and had to describe all the in-mouth sensations they felt while tasting all the samples (n = 5). The evaluators had important explanations about the concept of dominance, defined as the attribute associated with the sensation that draws the participant's attention, not necessarily the highest intensity attribute (Pineau, Bouillé, Lepage, Lenfant, Schlich, Martin, et al., 2012). The seven most-cited sensations were selected for the TDS analysis: sandy texture, softness, salty taste, spice flavor, firmness, gelatinous texture, and chia flavor. Moreover, the subjects went through a familiarization stage to introduce them to the sensations involved in the analysis. In this stage, each panelist was familiarized with the sensations involved in the TDS analysis, providing a reference relative to each sensation according to Table 2. More two sessions were used to introduce the Sensomaker program (Nunes & Pinheiro, 2012) and to define the evaluation time of the samples.

TDS analysis was conducted according to Pineau et al. (2009), in triplicate for each individual, totaling 36 evaluations per sample, using the data acquisition program Sensomaker (Nunes & Pinheiro, 2012) under the same conditions: total duration of analysis of 45 s with a ‘‘delay time” of 5 s. Moreover, the ‘‘no taste” option was included in the attributes list to allow the taster to indicate that no more sensation was perceived. The samples (approximately 6 g) were balanced and coded with 3-digit numbers drawn from a table of random numbers and presented in monadic order (Macfie, Bratchell, Greenhoff, & Vallis,1989).

2.10.2 Acceptance test and Check all that apply (CATA)

A total of 117 consumers that regularly consumed Bologna sausages were recruited among students and staff of the University of Campinas (Campinas, Brazil). The color, aroma, flavor, texture, and overall acceptability were evaluated using a structured nine-point hedonic scale, with 1 being extremely disliked and 9 extremely liked (Stone & Sidel, 1993). Purchase intention was also evaluated using a 5-point scale (1 = “would certainly not buy” to 5 = “would certainly buy”). A three-digit code has been assigned to the samples, which were evaluated by

each consumer in a monadic order, following a balanced design as described by Macfie et al. (1989) in individual cabins.

Table 2. References to familiarize the panellists in relation to the sensations involved in the TDS test of Bologna sausages.

Attribute	Definition	Reference
Sandy texture	Presence of small particles during mastication of Bologna sausages.	Garbanzo paste
Firmness	The force required for food compression between molar teeth. A firm product shows resistance to chewing.	Commercial mortadella from marketing
Softness	The force required for food compression between molar teeth. A soft product has no resistance to chewing.	Fresh Minas cheese
Gelatinous texture	Oral perception obtained by cutting the sample with teeth. A very gelatinous product makes noise when chewing and breaks into small pieces that do not come together to form a homogeneous mass.	Gummy bears (Fini)
Chia flavor	Characteristic flavor of chia seeds.	10 g of chia seed in 60 ml of mineral water after 30 minutes of seed hydration.
Spice flavor	Flavor perceived by a mixture of seasonings like garlic, onion, herbs and spices.	A 2.5% complete seasoning (Arisco) solution in mineral water at 25 ºC
Salty taste	Describes the primary taste produced by an aqueous solution of sodium chloride.	A 0.25% sodium chloride solution in mineral water at 25 ºC.

The participants tasted each sample and filled out the questionnaire. The first part of the questionnaire consumers is asked to first evaluate the attributes of the acceptance test on hedonic scale, and then to complete a check-all-that-apply (CATA) questionnaire with the 16 attributes. The sensory attributes of appearance (light pink color, shiny surface, dark pink color, light brown color), aroma (Bologna sausage aroma, rancid aroma), flavor (adequate amount of salt, Bologna sausage flavor, strange residual taste, little salty) and texture (succulent, soft texture, firm, rubber texture, little succulent, and sandy texture) were used to characterize the sensory profile of the Bologna sausages. Attributes were developed with point of departure on existing sensory literature on meat products (e.g. Jorge, Mendes, Auriema, Cazedey, Fontes, Souza, et al., 2015; Saldaña, Garcia, Selani, Haguiwara, Almeida, Siche, et al., 2018), and later

refined and modified based on qualitative tastings among the authors and collaborators. The consumers were asked to check all of the terms that they considered appropriate to describe each sample. The sensory terms were balanced within and across consumers, following William's Latin Square experimental design (Ares, Barreiro, Deliza, Giménez & Gámbaro, 2010).

2.11 Statistical analysis

The effects of the addition of chia mucilage and reduction of fat and phosphates on the physicochemical and technological properties of Bologna sausages was evaluated using a randomized complete block design. Three replicates were performed on three days. The results were analyzed using analysis of variance (ANOVA) with linear models (GLM – *General Linear Models)*, considering the treatments as a fixed effect, and the experiment replications as a random term ($n = 3$), using of the Statsoft. Inc. version 7 software (TIBCO Software Inc., California, USA). Tukey's test at 5% significance level ($P \leq 0.05$) was used to determine significant differences between treatments.

TDS curves of Bologna sausages were plotted according to the methodology proposed by Pineau et al. (2009), using the Sensomaker software (Nunes & Pinheiro, 2012). Two lines were drawn in the TDS graphical display: the “chance level” and the “significance level”. The “chance level” is the dominance rate that an attribute can obtain by chance and the “significance level” is the minimum value of this ratio considered significant. For this calculation, we used the confidence interval for a binomial proportion based on the normal approximation, according to Pineau et al., 2009. The CATA results were analyzed by frequency analysis of citations for each sensory term of each treatment using XLStat software (version 2018, Addinsoft, Paris, France), Cochran's Q test was performed to identify significant differences between treatments for each term assigned in CATA (Ares & Jaeger, 2013).

3. Results and discussion

3.1 Proximate composition, pH, and aw

Proximate composition analysis of the samples subjected to the different treatments revealed some significant differences ($P<0.05$) (Table 3). The moisture content was higher in samples containing MCP and MCG ($P<0.05$), because, due to the reduced fat content in these

samples, there was an adjustment of formulations with ice; other authors have also used this reformulation strategy (Paglarini et al., 2019). There were no significant differences ($P<0.05$) between the Bologna sausages with regards to the protein content. Lower ash contents were observed in case of treatments F1 and F2, which contained a reduced amount of fat and 2% chia mucilage (MCP and MCG, respectively). A fat content reduction of at least 31.5% was observed in samples containing chia mucilage and a reduction in phosphate levels of 34% was observed compared to the control (FC). However, the levels of chia mucilage (4% moisture, 12% protein, 25% lipids, and 6% ash, unpublished data) used for the various treatments in this study did not influence the proximate composition of the Bologna sausages.

The pH values of the Bologna sausages (Supplementary material 1) were similar, with only slight variations; the pH values (in day 0) ranged from 5.93 (FC) to 6.10 (F5). Samples containing chia mucilage had higher pH values, but these differences did not affect the final quality of the products; these results are consistent with those of other studies (Pintado, Herrero, Jiménez-Colmenero, & Ruiz-Capillas, 2016; Pintado, Herrero, Jiménez-Colmenero, Cavalheiro, & Ruiz-Capillas, 2018). At the end of the storage time (day 60), the pH ranged from 5.91 (F3) to 6.06 (F2 and F5). The aw values (Supplementary material 1) were higher in case of samples with reduced fat contents and higher moisture contents, as expected (0.98 in case of F1, F2, F5, and F6), and differed ($P<0.05$) from those of the control (0.975 in FC).

3.2 Emulsion stability

Phosphates are generally used in meat products to increase the water-holding capacity and improve the cooking yield, which are widely known as desirable characteristics for meat products (Pinton et al., 2019; Knipe, Rust, & Olson, 1990). Phosphates improve water and fat binding, since they act synergistically with sodium chloride, increasing the ionic strength of the meat system; therefore, they also improve the solubility of myofibrillar proteins, leading to the formation of a more stable meat gel with better binding properties in meat products such as Bologna sausages (Desmond, 2006). Hence, lowering the content of these additives, which have such essential properties, in meat products is challenging.

The results revealing the effects of phosphate reduction and the addition of MCP and MCG on emulsion stability in the samples are shown in Table 3. Fat reduction and the absence of phosphates (F1, F2, F3, and F4) increased ($P<0.05$) the percentage of liquid and fat release compared to control (FC); this indicates a reduction of the emulsion stability. Chia

mucilage, despite its high water-holding capacity (Muñoz et al., 2012), was not able to fully compensate for the absence of phosphates, especially in case of the treatments F1 and F2.

Table 3. Emulsion stability, chemical composition and color parameters of Bologna sausages elaborated with chia mucilage powder (MCP) and chia mucilage in gel (MCG).

Parameter	Days	FC	F1	F2	F3	F4	F5	F6	SEM*
Emulsion stability									
Liquid released (%)	0	0.76^{e}	13.38^{a}	11.87^{b}	7.46^{c}	7.34^{c}	2.76^{d}	2.69^{d}	0.580
Fat released (%)	0	0.05^{d}	1.02^{a}	0.90^{a}	0.63^{b}	0.61^{b}	0.21^{c}	0.27^{c}	0.044
Chemical composition									
Moisture (%)	0	62.45^{e}	$68.35^{b,c}$	69.28^{a}	67.55^{d}	$68.15^{c,d}$	$68.91^{a,b}$	$67.87^{c,d}$	0.140
Protein (%)	0	16.78^{a}	16.81^{a}	16.76^{a}	16.94^{a}	16.90^{a}	16.96^{a}	16.68^{a}	0.236
Fat (%)	0	16.69^{a}	10.80^{c}	10.48^{c}	11.44^{b}	$10.62^{b,c}$	10.89^{c}	$10.59^{b,c}$	0.221
Ash (%)	0	2.73^{a}	2.36^{e}	2.34^{d}	2.51^{d}	2.48^{c}	$2.71^{a,b}$	2.63^{b}	0.033
Sodium tripolyphosphate (%)	0	0.59^{a}	$0.38^{b,c}$	0.36^{c}	0.37^{c}	$0.39^{b,c}$	$0.47^{a,b,c}$	$0.50^{a,b}$	0.038
Color parameters									
L*	0	$63.97^{a,A}$	$58.30^{c,d,e,B}$	$60.17^{b,A,B}$	$57.75^{d,e,B}$	$58.60^{c,d,A,B}$	$58.98^{c,C}$	$57.43^{e,B,C}$	0.210
	15	$63.81^{a,A}$	$58.73^{c,d,A,B}$	$60.11^{b,A,B}$	$58.51^{c,d,A,B}$	$58.06^{d,B}$	$59.49^{b,c,B,C}$	$58.21^{d,A,B}$	0.213
	30	$64.11^{a,A}$	$58.81^{c,d,A,B}$	$60.92^{b,A}$	$58.41^{c,d,A,B}$	$58.96^{c,A}$	$59.32^{c,B,C}$	$57.55^{d,B,C}$	0.233
	45	$63.92^{a,A}$	$59.36^{c,A}$	$59.43^{c,B}$	$58.84^{c,A}$	$58.98^{c,A}$	$60.70^{b,A}$	$59.10^{c,A}$	0.197
	60	$63.96^{a,A}$	$58.04^{d,e,B}$	$59.32^{b,c,B}$	$58.35^{c,d,A,B}$	$58.37^{c,d,A,B}$	$60.07^{b,A,B}$	$56.90^{e,C}$	0.239
a*	0	$9.53^{a,A}$	$7.71^{d,e,B}$	$8.08^{b,c,d,B}$	$8.43^{b,A}$	$8.19^{b,c,A}$	$7.89^{c,d,e,B}$	$7.45^{e,C}$	0.100
	15	$9.22^{a,A,B}$	$8.21^{b,c,A,B}$	$8.41^{b,A,B}$	$7.97^{b,c,B}$	$8.22^{b,c,A}$	$8.08^{b,c,B}$	$7.79^{c,C}$	0.073
	30	$9.46^{a,A}$	$8.59^{a,b,A}$	$8.55^{a,b,A,B}$	$7.98^{b,B}$	$8.51^{a,b,A}$	$8.95^{a,b,A,B}$	$8.90^{a,b,B}$	0.123
	45	$9.25^{a,A,B}$	$8.01^{c,A,B}$	$8.89^{a,b,A}$	$8.10^{c,A,B}$	$8.36^{b,c,A}$	$7.87^{c,B}$	$7.82^{c,C}$	0.082
	60	$8.96^{b,c,B}$	$7.89^{d,A,B}$	$8.43^{c,d,A,B}$	$8.07^{c,d,A,B}$	$8.08^{c,d,A}$	$9.54^{b,A}$	$10.57^{a,A}$	0.151
b*	0	$13.52^{e,A}$	$14.55^{d,B}$	$14.92^{c,d,B}$	$15.97^{b,B}$	$16.11^{a,b,B}$	$15.25^{c,A}$	$16.55^{a,A}$	0.131
	15	$13.41^{d,A,B}$	$14.59^{c,B}$	$15.03^{c,B}$	$16.05^{a,b,A,B}$	$16.38^{a,A,B}$	$15.65^{b,A}$	$16.28^{a,A}$	0.140
	30	$13.40^{c,A,B}$	$14.79^{b,B}$	$15.25^{b,B}$	$16.57^{a,A}$	$16.93^{a,A}$	$13.71^{c,C}$	$15.43^{b,B}$	0.180
	45	$13.07^{c,B}$	$14.84^{b,B}$	$15.88^{a,A}$	$16.43^{a,A,B}$	$16.45^{a,A,B}$	$14.81^{b,A,B}$	$16.30^{a,A}$	0.162
	60	$13.42^{d,A,B}$	$15.56^{a,A}$	$15.27^{a,b,B}$	$16.07^{a,A,B}$	$16.11^{a,B}$	$14.18^{c,d,B,C}$	$14.65^{b,c,C}$	0.144
Whiteness	0	$60.08^{a,A}$	$55.16^{c,A,B}$	$56.70^{b,A,B}$	$54.05^{d,e,A}$	$54.83^{c,d,A}$	$55.53^{c,C}$	$53.72^{e,B,C}$	0.264
	15	$60.32^{a,A}$	$55.46^{b,c,d,A,B}$	$56.55^{b,A,B}$	$54.81^{c,d,e,A}$	$54.23^{e,A}$	$55.82^{b,c,B,C}$	$54.48^{d,e,A,B}$	0.265
	30	$60.54^{a,A}$	$55.39^{c,d,A,B}$	$57.18^{b,A}$	$54.52^{d,e,A}$	$54.79^{c,d,e,A}$	$56.11^{b,c,B,C}$	$53.93^{e,B,C}$	0.287
	45	$60.52^{a,A}$	$55.99^{c,A}$	$55.53^{c,B}$	$54.94^{c,A}$	$55.02^{c,A}$	$57.27^{b,A}$	$55.28^{c,A}$	0.253
	60	$60.51^{a,A}$	$54.55^{c,B}$	$55.74^{b,B}$	$54.63^{c,A}$	$54.63^{c,A}$	$56.52^{b,B}$	$53.27^{d,C}$	0.291

*SEM- Standard error of the mean. ^{a,b,c,d,e}Mean values within the same line horizontally followed by the same lowercase letters did not show any significant difference ($P > 0.05$) by Tukey's test. ^{A,B,C}Mean

values within the same column followed by the same capital letters did not show significant difference (P>0.05) by Tukey's test. [1] For treatments denominations, see Table 1.

However, the liquid and fat release in treatments containing a higher content of chia mucilage, 4% (F3 - 4% MCP and F4 - 4% MCG) was low, regardless of the form of chia mucilage used (powder or gel);even so, the liquid and fat release observed in these samples was still much higher than that observed in case of the control sample (FC), which contained 0.5% phosphate. With regards to the incorporation form of the mucilage in the products, the comparison of F1 (2% MCP) and F2 (2% MCG), revealed a lower liquid release in case of F2, which suggests that the MCG provides a better stability. The treatments F5 and F6, both of which contained 50% lower phosphate levels, showed similar results, regardless of the final MCG content (2% or 4%, respectively). The stability of the meat emulsion was significantly improved in these samples, with a considerable decrease of the liquid and fat release.

3.3 Color parameters

The color of meat products is crucial for the acceptance and purchase decisions in case of meat products; it is the first parameter to be evaluated (Troy & Kerry, 2010). The nitrosohemichrome pigment, which is usually perceived as a pink-colored pigment, is developed in meat products by the addition of nitrites and/or nitrates after cooking (Aberle et al., 2001). In the reformulation of meat products, many natural ingredients, such as flours, certain types of fibers, and healthier oils, have different colors and can alter the appearance of the products (Fernández-López et al., 2019; Wolfer, Acevedo, Prusa, Sebranek, & Tarte, 2018). In this study, instrumental color evaluation of the Bologna sausages (Table 3) showed that the addition of the chia mucilage affected the L*, a*, and b* color parameters.

At the initial storage period (day 0), the luminosity (L*) was lower (P<0.05) in case of all treatments containing chia mucilage and reduced fat contents than in the control (FC); additionally, a darker tone was perceived in these treatments. This darker color is due to the appearance of the chia mucilage (instrumental color values were 51.89 ± 0.52 for L*; 5.10 ± 0.24 for a*, and 19.98 ± 0.31 for b*, data not shown), since the seeds were subjected to a strong pressure during the extraction process to separate the mucilage, and a part of the outer coating of the seeds may have been incorporated into the mucilage. Similarly, the a* values were significantly lower (P<0.05) in the formulations containing the chia mucilage; there were no significant differences between the a* values of these samples, regardless of the final MC

content (2% and 4%) or the incorporation form of the mucilage (powder or gel). In contrast, the b* values were lower in case of samples containing 2% chia mucilage (F1, F2, and F5), but still significantly higher ($P<0.05$) than the control sample (FC). These results have also been reported by Pintado et al., 2016 and Fernández-López et al., 2019, via studies that assessed the effects of chia flour and a chia co-product derived from oil extraction, respectively, on meat products and showed lower lightness and higher yellowness values in samples containing these components.

With regards to the storage period, the control Bologna sausage (FC) was clearer, with higher values of L* and lower values of b*, compared to the samples containing chia mucilage throughout the evaluation period (60 days). In general, the luminosity was stable in case of the reformulated treatments, as the values at the beginning and the end of the evaluation period were similar, except in case of treatment F5, which showed increased L* values at the 45- and 60-day time of storage. Similar values were also found for the parameters a* and b* in treatments F1–F4 at the beginning and at the end of the evaluated period, except in case of F1, which showed increased a* values at the 60-day time. Treatments F5 and F6 showed an atypical behavior with regards to the color parameters, with a significant increase ($P<0.05$) of the a* values and a decrease of the b* values at the 60-day time. One hypothesis for explaining this phenomenon is that there were greater water and fat losses in these samples at the end of the storage period, as observed by the exudation or drip in the packages, leading to an increase in the concentration of the related pigments in these products.

3.4 Texture profile analysis (TPA)

The reformulation strategies used in this study had significant effects ($P<0.05$) on the texture properties of the Bologna sausages (Table 4). Regardless of the absence or reduction of phosphates, the samples containing chia mucilage and reduced fat contents were less firm and less chewable on day 0, except the case for treatment F3 (4% MCP), which showed the same hardness as the control (FC). The decrease in the hardness and chewiness of these samples is consistent with previous studies in which the fat content was reduced and the water content was increased, while keeping the protein content constant (Pietrasik & Janz, 2010; Saengphol & Pirak 2018; Jiménez-Colmenero, Carballo, & Solas, 1995). With regards to the storage time, hardness and chewiness showed similar behaviors, with a significant increase ($P<0.05$) at the end of the storage period (60 days). Andrés, García, Zaritzky, & Califano (2006) have attributed

the increase of hardness of meat products during storage to changes related to losses or purging of the products.

Intriguing results were obtained for the elasticity and cohesiveness (Table 4) of the Bologna sausages, and the effects of the reduction/absence of phosphates, in association with the incorporation of chia mucilage, were more evident. Significantly equal values of elasticity ($P>0.05$) were found on day 0 for the control (FC) and the treatments F2 and F5; both F2 and F5 contained 2% MCG, but F5 contained phosphates (0.25%), while F2 did not. Lower values of elasticity ($P<0.05$) were found in case of samples containing 2% MCP (F1) and 4% chia mucilage (F3 - 4% MCP, F4 - 4% MCG, and F6 - 4% MCG), which shows that the mucilage incorporation form, as well as concentration, had an impact on this parameter.

Table 4. Texture parameters of Bologna sausages elaborated with chia mucilage powder (MCP) and chia mucilage in gel (MCG).

	Days	FC[1]	F1	F2	F3	F4	F5	F6	SEM*
Hardness (N)	0	$14.25^{a,D}$	$12.19^{c,C}$	$12.93^{b,C}$	$14.15^{a,B}$	$13.00^{b,D}$	$10.05^{e,B}$	$11.44^{d,B}$	0.180
	15	$14.87^{a,C,D}$	$13.90^{b,c,B}$	$13.27^{d,B,C}$	$14.43^{a,b,B}$	$13.44^{c,d,C}$	$10.09^{f,B}$	$11.39^{e,B}$	0.207
	30	$15.07^{a,C}$	$14.08^{b,B}$	$13.48^{c,B}$	$14.39^{b,B}$	$13.36^{c,C,D}$	$10.20^{e,B}$	$12.48^{d,A}$	0.192
	45	$16.11^{a,B}$	$14.39^{c,A,B}$	$14.76^{b,c,A}$	$15.94^{a,A}$	$15.08^{b,A}$	$11.10^{e,A}$	$12.80^{d,A}$	0.215
	60	$17.46^{a,A}$	$14.87^{b,A}$	$14.69^{b,A}$	$14.51^{b,B}$	$14.33^{b,B}$	$10.97^{d,A}$	$12.76^{c.A}$	0.241
Springiness	0	$0.914^{a,C}$	$0.894^{b,C}$	$0.911^{a,A}$	$0.892^{b,C}$	$0.895^{b,B}$	$0.910^{a,B}$	$0.900^{b,B}$	0.001
	15	$0.916^{a,C}$	$0.895^{b,C}$	$0.902^{b,B}$	$0.897^{b,B,C}$	$0.896^{b,A,B}$	$0.917^{a,A,B}$	$0.904^{b,B}$	0.001
	30	$0.925^{a,B}$	$0.899^{c,B,C}$	$0.913^{b,A}$	$0.901^{c,A,B,C}$	$0.903^{c,A,B}$	$0.920^{a,b,A}$	$0.916^{b,A}$	0.001
	45	$0.928^{a,A,B}$	$0.910^{b,c,A}$	$0.916^{b,A}$	$0.904^{c,A,B}$	$0.903^{c,A,B}$	$0.916^{b,A,B}$	$0.915^{b,A}$	0.001
	60	$0.932^{a,A}$	$0.907^{d,A,B}$	$0.917^{b,c,A}$	$0.909^{c,d,A}$	$0.906^{d,A}$	$0.924^{a,b,A}$	$0.918^{b,c,A}$	0.001
Chewiness (N)	0	$10.32^{a,C}$	$8.64^{d,C}$	$9.19^{c,B}$	$9.85^{b,B}$	$9.31^{c,B}$	$7.30^{e,C}$	$8.40^{d,B}$	0.122
	15	$10.44^{a,C}$	$9.65^{b,c,B}$	$9.30^{c,B}$	$9.87^{b,B}$	$9.46^{b,c,B}$	$7.82^{d,B}$	$8.36^{d,B}$	0.114
	30	$10.73^{a,C}$	$9.79^{b,c,B}$	$9.50^{c,B}$	$10.05^{b,B}$	$9.62^{b,c,B}$	$8.41^{d,A}$	$10.11^{b,A}$	0.094
	45	$11.78^{a,B}$	$10.22^{d,A}$	$10.51^{c,d,A}$	$11.03^{b,A}$	$10.88^{b,c,A}$	$8.34^{e,A}$	$10.04^{d,A}$	0.133
	60	$12.97^{a,A}$	$10.28^{c,A}$	$10.45^{b,c,A}$	$10.82^{b,A}$	$10.92^{b,A}$	$8.35^{d,A}$	$10.09^{c,A}$	0.166
Cohesiveness	0	$0.817^{a,A}$	$0.789^{c,A,B}$	$0.791^{c,A,B}$	$0.790^{c,A,B}$	$0.785^{c,A,B}$	$0.805^{b,C}$	$0.800^{b,B}$	0.001
	15	$0.821^{a,A}$	$0.778^{b,C}$	$0.786^{b,B}$	$0.787^{b,A,B}$	$0.787^{b,A,B}$	$0.819^{a,A,B}$	$0.818^{a,A}$	0.002
	30	$0.819^{a,b,A}$	$0.783^{d,B,C}$	$0.791^{c,d,A,B}$	$0.788^{c,d,A,B}$	$0.794^{c,A}$	$0.824^{a,A}$	$0.814^{b,A}$	0.002
	45	$0.820^{a,A}$	$0.794^{b,A}$	$0.792^{b,A,B}$	$0.781^{c,B}$	$0.783^{c,B}$	$0.815^{a,B}$	$0.814^{a,A}$	0.002
	60	$0.821^{a,A}$	$0.787^{b,A,B}$	$0.795^{b,A}$	$0.794^{b,A}$	$0.792^{b,A}$	$0.818^{a,A,B}$	$0.815^{a,A}$	0.002

*SEM- Standard error of the mean. ^{a,b,c,d,e,f}Mean values within the same line horizontally followed by the same line horizontally followed by the same lowercase letters did not show any significant difference (P > 0.05) by Tukey's test. ^{A,B,C}Mean values within the same column followed by the same capital letters did not show significant difference (P>0.05) by Tukey's test. [1] For treatments denominations, see Table 1.

According to Han & Bertram (2017), it is possible that the addition of fibers in meat systems affects the formation of the protein–water or protein–protein networks during gelation, which in turn, decreases the gel strength of the product, thereby weakening the bonds. This decrease in elasticity has also been observed by Fernández-López et al. (2019), when they added 3% chia flour in Frankfurter sausages.

Probably, the addition of 4% chia mucilage was sufficient to affect the elasticity and, also cohesiveness of the Bologna sausages. The FC was the most cohesive at day 0 and differed ($P<0.05$) from that of the other treatments. However, the treatments F5 and F6 (2% and 4% MCG, respectively; both contained 0.25% phosphates) were more cohesive ($P<0.05$) than those without phosphates (F1, F2, F3, and F4), which again shows that these additives are crucial for the solubilization of myofibrillar proteins and stabilization of meat emulsions (Desmond, 2006; Barbut, Maurer, & Lindsay, 1988). Lower cohesiveness and elasticity represent a serious technological problem that can affect the quality characteristics of emulsified meat products, such as the sliceability (Pinton et al., 2019). From day 15, the cohesiveness of the treatments F5 and F6 was identical ($P>0.05$) to that of the control (FC). Similar to the case for hardness and chewiness, the elasticity and cohesiveness increased over time. The mechanisms underlying this increasing trend of texture-related parameters during storage are still poorly understood, and a similar behavior has been reported by Pintado et al. (2016) in Frankfurter sausages containing chia flour and olive oil.

3.5 Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) was used to assess changes in the protein structure and fat particles of the Bologna sausages, caused by the reduction of fat and phosphates and the addition of chia mucilage (Figure 1). The scale bar in each CLSM image indicates 250 μm. Fat is observed by a red color, staining with Nile Red, and meat proteins and MC fibers are observed by a green color, staining with fluorescein isothiocyanate (FITC). The images elucidate the interactions that occurred in the emulsified meat system, which has a complex nature, and confirmed the texture and stability differences found in the products.

Comminution, in combination with the increase in ionic strength, and subsequent heating are processes used to manufacture emulsified meat products; they drastically change the structure of meat systems. In these products, such as Bologna sausages, high amounts of myofibrillar proteins are extracted, which, when heated, create a dense protein network, i.e., a gel, which holds an adequate amount of water (Tornberg, 2005). Phosphates act very efficiently

in these systems, increasing the solubility of these proteins, as shown in the image for the control treatment FC (Figure 1). This sample had a cohesive protein structure with well-entangled fat droplets, but these droplets were unevenly dispersed by the protein network, with the mean particle sizes showing a wide variation. Changes in structure were observed when chia mucilage was added to the system. In treatments F1 (2% MCP) and F2 (2% MCG), both of which did not contain phosphates, the protein structures still showed a certain level of cohesion, but the fat particles, which had a well-defined appearance and were smaller, were "looser", i.e., less bound and entangled in the protein matrix, especially in case of F1. This characteristic is shown in the image for F1 and can be directly related to the lower emulsion stability found in case of this treatment (Table 3). On the other hand, F5, which also contained 2% MCG, but with 0.25% phosphates, showed a more compact organization, with fat droplets that were better integrated and aggregated to the protein matrix.

Another important change in the evaluated meat systems occurred when the amount of chia mucilage added was doubled, i.e., in case of the treatments F3 (4% MCP), F4 (4% MCG), and F6 (4% MCG). The protein structures in F4 and F6 were spongier, less continuous and cohesive, and more heterogeneous, with small fat droplets being located in these matrices in the form of small clumps. Sample F6 contained 0.25% phosphate, which does not seem to have the same effect on the formation of a denser protein matrix, as that observed in case of F5. Possibly, a higher chia mucilage concentration may have affected the formation of protein films around the fat droplets and the interactions of the protein–fat network in the samples. Other authors have reported that the addition of dietary fiber affects this structure in meat products (Han, Clausen, Christensen, Vossen, Hecke, & Bertram, 2018).

Moreover, the chia mucilage, especially at higher concentrations, may have had an atypical behavior in the aqueous medium of the meat system, which has a high ionic strength. Munõz et al. (2012) have observed that the water absorption ability of chia mucilage is strongly dependent on the salt concentration; as observed in case of emulsified meat systems, its water-holding capacity decreases at salt concentrations above 0.5%. The action of chia mucilage in complex meat systems still requires further elucidation, since it is influenced by several components of this system, such as additives, connective tissue, fat, and meat fibers, in an environment subject to variations in ionic strength, pH, and temperature.

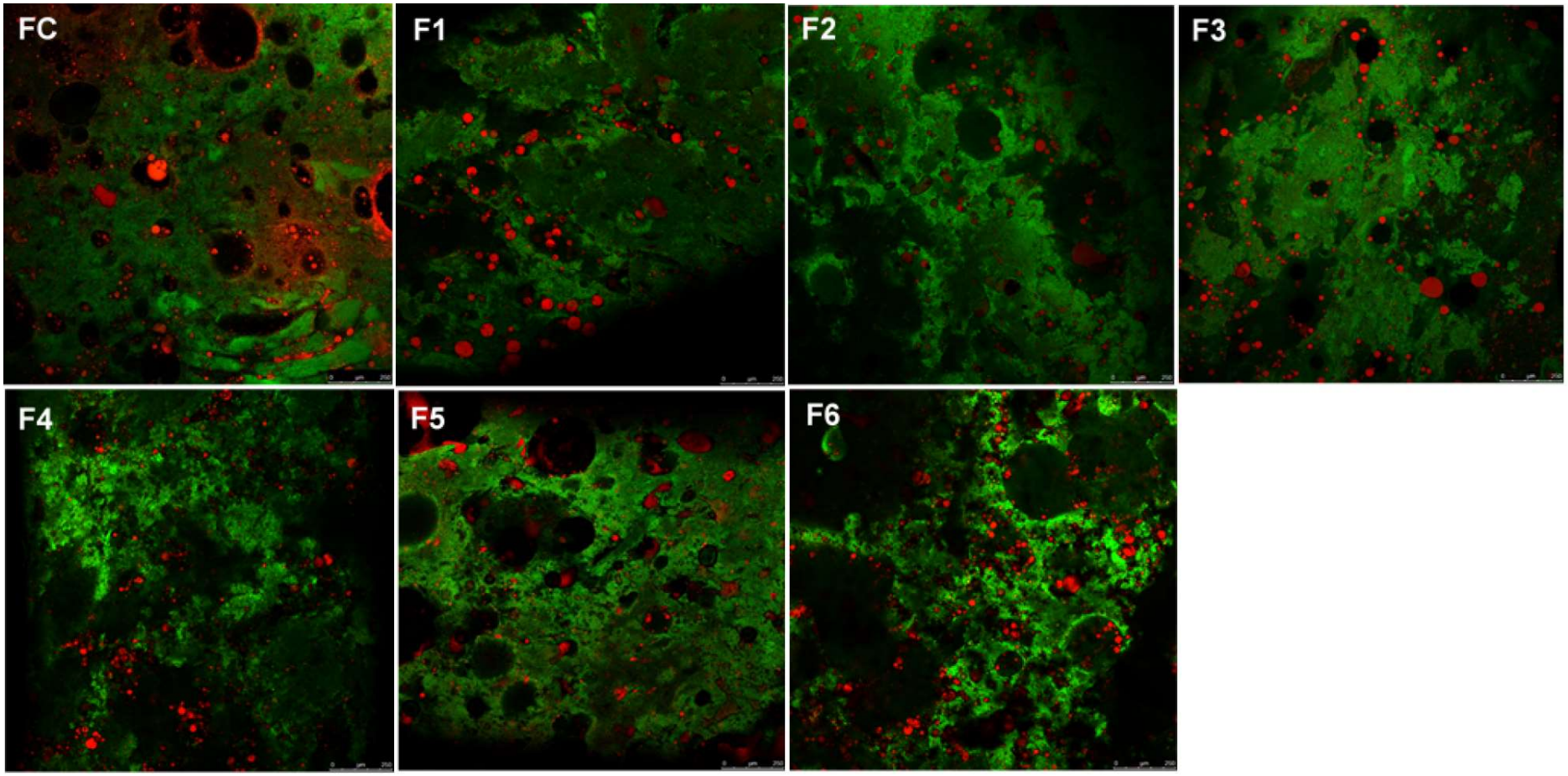

Fig 1. Confocal scanning laser microscopy images of Bologna sausages elaborated with chia mucilage powder (MCP) and chia mucilage in gel (MCG). The green, red and black represent protein, oil and air/water, respectively. Magnification: 10 x; Scale bar: 250 μm. For treatments denominations, see Table 1.

3.6 Low field NMR analysis

The low field NMR technique is a useful tool for exploring the intrinsic characteristics of water and measuring quantities such as T_2 relaxation times that are sensitive to molecular motion. It is possible to estimate the mobility and structural properties of different fractions of water molecules, which is influenced by structural constraints within the meat matrix (Bertram, Purslow, & Andersen, 2002; Han, Wang, Xu, & Zhou, 2014). The NMR signal decay for all Bologna sausages samples could be fitted into two distinct exponential separate peaks (T_{21} centered at approximately 40-50 ms and T_{22} centered at about 300-400 ms), as shown in Fig. 2a, which provide the information on how the water is distributed in the sausages.

Comparing the T_2 relaxation times found here with previously reported studies (Hjelm et al., 2019; Møller et al., 2011), it suggests that the T_{21} component reflects the water immobilized in the protein structure, while T_{22} refers to the intermyofibrillar water located between the fiber bundles which interacts most weakly with the charged groups. Although studies demonstrate the potential of chia mucilage to bind to water (Muñoz et al., 2012), to our best knowledge no previous research has elucidated how the inclusion of MC as an ingredient in an emulsified meat product influences the mobility and intrinsic distribution of water. Intriguingly, distributed exponential fitting analysis of T_2 relaxation data on the Bologna sausages revealed a pronounced effect of MC on the intrinsic water mobility and distribution as the inclusion of MC at concentrations of 4% (F3, F4, and F6) resulted in the presence of a distinct water population located in the range between 300 and 325 ms (Fig. 2b). In these treatments, there was a considerable decrease in relaxation times, with faster relaxation rates for both populations, T_{21}, and T_{22}. These results demonstrated that the protons of these samples (F3, F4, and F6) had more restricted mobility.

When the phosphate reduction is evaluated, the mobility of the water molecules, represented by the T_{21} and T_{22} relaxation times, did not show greater differences when comparing the control treatment (FC) with 0.5% of phosphates and F1 (2% MCP), and F2 (2% MCG), without addition of phosphates (Fig. 2b). The reduction of fat and phosphate content may have caused reorganization of myofibrillar water into meat product structure. However, the addition of chia mucilage probably made this change less abrupt, always maintaining the proportions of both population (T_{21} and T_{22}) around 50% (Fig. 2c).

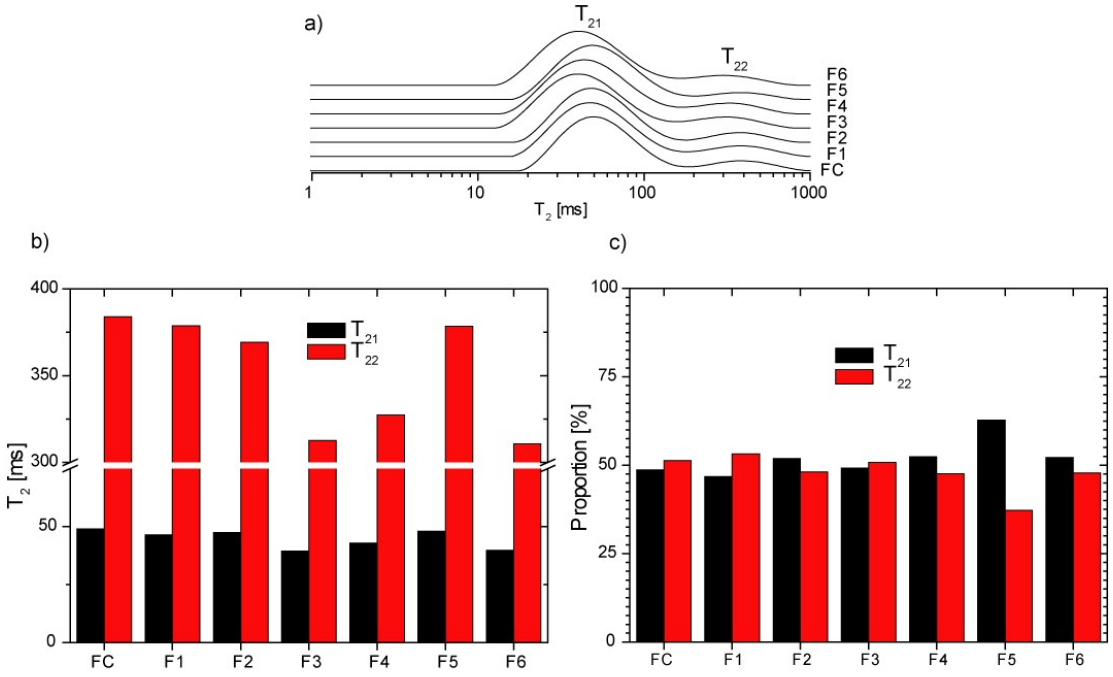

Fig 2. a) Distributions of T_2 relaxation times for Bologna sausages samples with two populations of water referred to as T_{21} and T_{22}. b) T_2 center relaxation times and c) peak areas changes for all treatments, respectively. For treatments denominations, see Table 1.

3.7 Sensory Evaluations

3.7.1 Temporal Dominance of Sensation (TDS)

Chia derivatives have been reported in previous literatures as functional ingredients with different physiological properties. As an alternative ingredient to reduce animal fat in this study, it is relevant to investigate its impact on the sensory properties of meat systems. The main objective of the TDS analysis is identifying the attribute perceived as "dominant" throughout food consumption. "Dominant" is defined as the sensation that captures one's attention or the most striking perception but may not be necessarily the most intense one (Pineau et al., 2009).

Figure 3 shows the TDS plots for the five treatments selected in this study. The sensory profiles of the samples are related to the attributes of sandy texture, softness, salty taste, spicy flavor, firmness, gelatinous texture, and chia flavor during 45 s of evaluation.

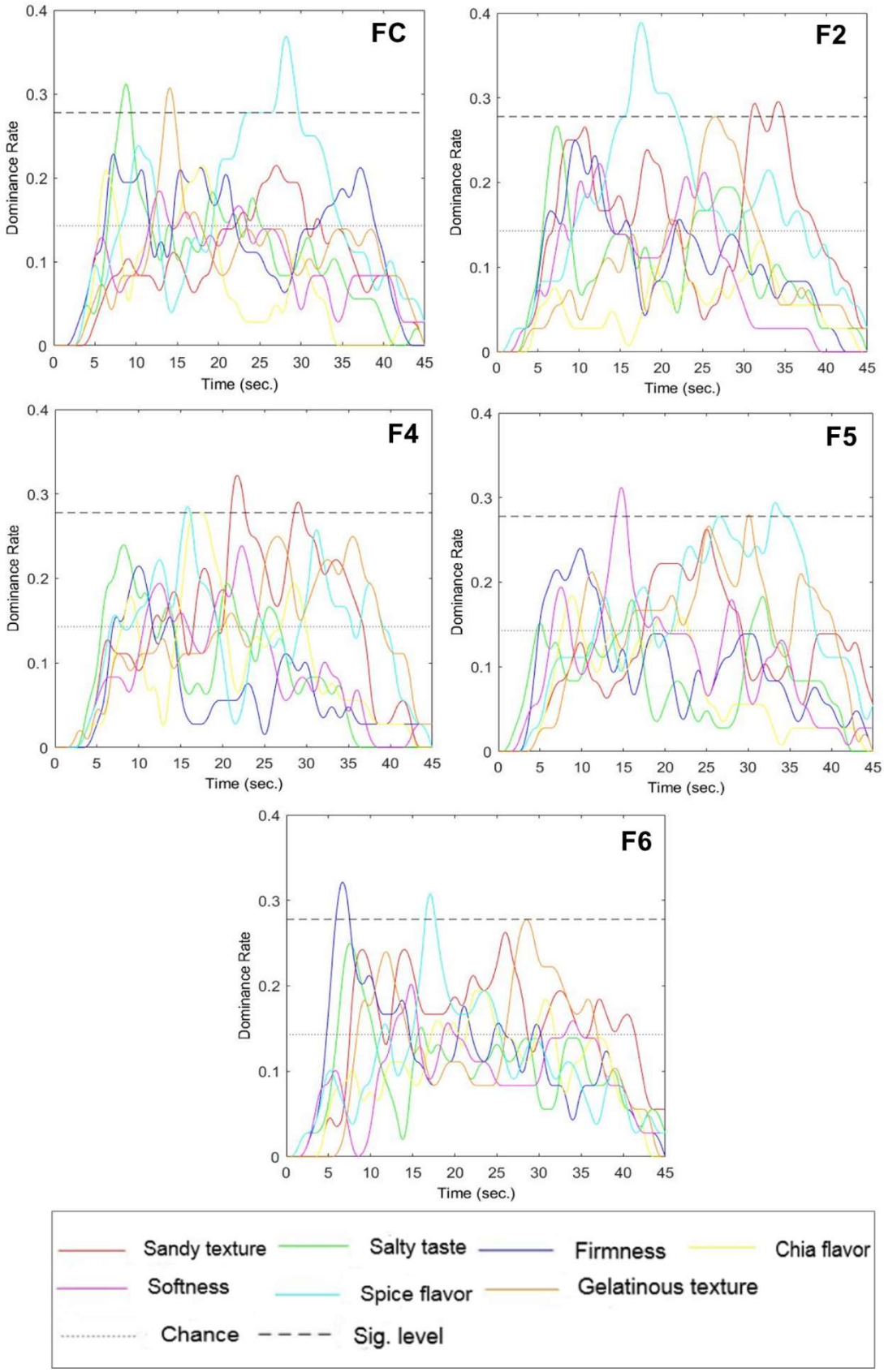

Fig 3. Smoothed TDS curves for the five treatments of selected Bologna sausages: FC, F2, F4, F5, and F6. The x-axis indicates time in seconds. The y-axis shows the dominance rate. For treatments denominations, see Table 1.

Salty taste was the first dominant attribute in the FC (with a maximum dominance rate at 8.8 s), followed by gelatinous texture and spicy flavor. The salty taste attribute was only dominant in the control treatment (FC), despite all treatments having the same salt content. However, other formulations did not contain phosphates (F2 and F4) or had a 50% reduction in the fat content (F5 and F6), which could explain this salty taste perception of the evaluators in case of the FC, since sodium tripolyphosphate has about 30% sodium. In emulsified meat products, the gelatinous texture refers to the gel that is formed in these products after the cooking step, which is enhanced by the presence of phosphates (Aberle et al., 2001).

Among the reformulated treatments, F2 had a spicy flavor, gelatinous texture, and sandy texture (significantly dominant attribute at two time periods) as dominant attributes, while the attributes spicy flavor, chia flavor, and sandy texture were dominant in case of F4. For the treatment F2, the volunteers were able to perceive the gelatinous texture as a dominant attribute, even with a reduced fat content and the absence of phosphates, providing important information about the role of MCG in the texture of the products. Due to its structure, chia mucilage acts as a soluble fiber and is known to have a high water-holding capacity and promote gel formation and the stabilization of meat emulsions (Muñoz et al., 2012; Timilsena, Adhikari, Kasapis, & Adhikari, 2016). However, at the end of the evaluation, the sandy texture attribute was significant, with a maximum dominance rate at 34 s, which shows that the chia mucilage texture, which was a little rougher and thicker due to the extraction method, affected the final texture perception of the samples. In case of treatment F4, which contained double the MCG concentration (4%), the chia flavor attribute was perceived as dominant, with a maximum dominance rate at 16 s, followed by sandy texture, with a maximum dominance rate at 21.7 s. Therefore, higher MCG concentrations had an impact on the taste, as the chia taste was perceived, and on the sandy texture, which was perceived sooner during the evaluation of F4 by the volunteers, compared to F2.

Other promising results on the role of phosphates in texture perception in meat products refer to treatments F5 and F6, which had a 50% reduction in the sodium tripolyphosphate content and the same MCG concentrations as F2 and F4 (2% and 4%, respectively). The volunteers did not assign the sandy texture attribute as dominant in case of samples F5 and F6, although these samples had the same MCG amounts as F2 and F4. The hypothesis for explaining this phenomenon is that phosphates, in association with MC, promoted the solubilization of myofibrillar proteins during the formation and stabilization of the meat gel; consequently, the gelatinous texture attribute was dominant, and the sandy texture attribute was not significantly perceived. In addition, in the evaluation of F6, firmness was the

first dominant attribute, with a maximum dominance rate (0.32) at 6.7 s, followed by spicy flavor and gelatinous texture. Possibly, the presence of higher MCG (4%) concentrations, in combination with phosphates, contributed to this dominant perception of firmness in the products, but this evaluation does not agree with the results of the instrumental texture measurements (Table 3) in this study.

The evaluation of meat products by the TDS methodology was adequate to compare several attributes over time and provided essential information on the characteristics perceived in products reformulated using a pioneer approach to remove and reduce the phosphate and fat contents by the addition of chia mucilage. According to Pineau et al. (2009), the evaluator should characterize the main temporal aspects of the products, simultaneously integrating all perceived sensations. However, Schlich (2017) makes an important consideration regarding the limitations still found in this method, since the definition of dominance is based on what attracts the attention of the evaluator at a given moment. However, the reasons for an attribute to capture attention vary between individuals and between product types; the factors influencing this aspect include the perception of a new attribute, the extinction of the perception of an old attribute, a cognitive search for novelty, and a sudden variation in the perceived intensities. Therefore, according to Schlich (2017), dominance should be further elucidated and defined.

3.7.2 Acceptance test and the check-all-that-apply (CATA) questionnaire

The results of the consumer acceptance test and the evaluation of the CATA questionnaire are shown in Figure 4. There were no significant differences ($P>0.05$) between the control treatment (FC) and the treatments F2 and F5 (both containing 2% MCG) with regards to aroma, taste, and texture attributes, with evaluation scores above 6.5, which correspond to evaluations between “slightly liked” and “moderately liked”. In general, Bologna sausages containing 2% MCG were well accepted, and the 50% fat reduction and absence of phosphates did not negatively influence the consumers' evaluation of these samples. These results can be directly related to the descriptive terms that discriminate these samples in the CATA questionnaire (left, Figure 4), with the term Bologna sausage flavor being associated with treatment F5, and the term succulent being associated with treatment F2. On the other hand, samples containing 4% MCG (F4 and F6) showed the worst sensory evaluation results for the attributes of taste, texture, and overall acceptance, and differed significantly ($P<0.05$) compared to the FC.

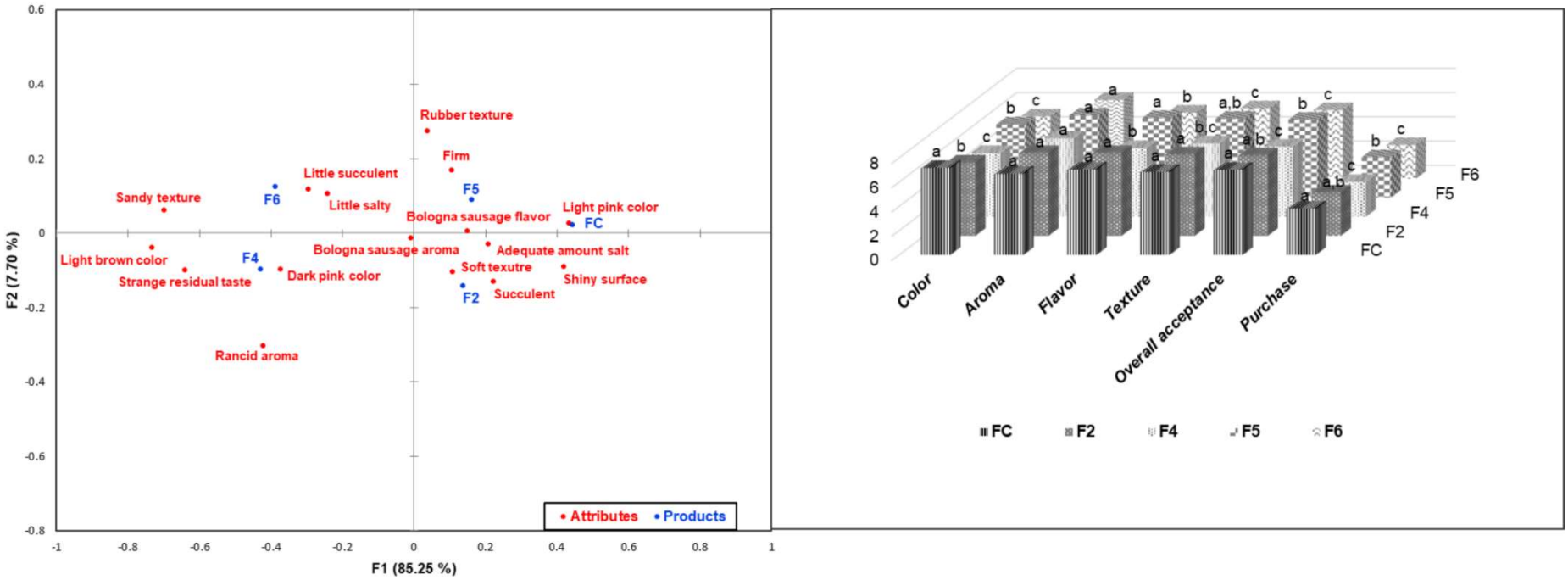

Fig 4. Representation of the samples and the terms in the first and second dimensions of correspondence analysis on data questions check-all-that-apply (CATA) (left) and results of consumer test (right) of selected Bologna sausages treatments: FC, F2, F4, F5, and F6. [a,b,c]Same letters in the direction of each attribute did not show significant differences (P >0.05) by the Tukey test between treatments. For treatments denominations, see Table 1.

F4 was characterized by the term strange residual taste, which can be related to the dominant chia flavor attribute found by the evaluators in the TDS evaluation of this sample. Furthermore, F6 was characterized by the negative term little succulent, which shows that the concentration of MCG (4%) affected the texture of the products, as can also be observed in the instrumental evaluation (TPA) (Table 4).

With regards to the color attributes, all treatments differed ($P<0.05$) from the control, and this evaluation was consistent with the lower values of the color parameters L* and a* and the higher values of b* (Table 3). The instrumental color evaluation had already provided information on the technological limitations in the reformulated Bologna sausages, and these characteristics were confirmed by the sensory evaluations. The sample containing 4% MCG (F4) was characterized by the terms light brown color and dark pink color; this treatment showed the worst acceptance by the evaluators for this attribute. These results have also been reported by Fernández-López et al. (2019) for Frankfurters containing 3% of a chia co-product, with lower scores for color, taste, and overall acceptance attributes.

Treatment F2 showed the best sensory evaluation results; compared to FC, it was also satisfactory with regards to the attributes of overall acceptance, purchase intention, texture, flavor, and aroma. Although treatment F5 showed good sensory evaluation results and contained 0.25% of phosphates, F2, which contained no phosphates, was found to be better. This result is very interesting and promising since the presence of MCG (2%) could compensate for the absence of these additives, which are considered highly important for the meat industry.

4. Conclusion

The results show that the addition of 2% MCG in the total absence of phosphates and with 50% fat reduction was effective and provided satisfactory results in Bologna sausages, except for the color attribute, which is a technological challenge that still needs to be surpassed.

The hypothesis of this study regarding the two incorporation forms of chia mucilage and their possible effects on the properties of the products was confirmed. The gel form provided better stability and texture parameters (closer to the control) at concentrations of up to 2%; no differences were found in case of samples containing higher concentrations of MCG. The sensory performance of the treatment containing 2% MCG without phosphates was comparable to that of the control treatment, with differences only for the color attribute. In addition, macrostructural changes in the Bologna sausages were noticeable at chia mucilage

concentrations of 4%. This concentration led to changes in the protein structure and fat binding in the products, which indicates the formation of a more weakly bound protein network and the presence of less entangled fat droplets in this matrix. Understanding the causes of such behavioral differences and the interactions between meat proteins and chia mucilage is essential for the development of healthier meat products; this study has shown very favorable prospects for the reduction of fats and phosphates in meat products. For the first time, this study has provided information on the performance of chia mucilage as an alternative to phosphates in Bologna sausages with reduced fat contents, and has highlighted the main effects of chia mucilage on their technological, sensory, and microstructural properties.

Declarations of interest

The authors declare that they have no conflict of interest.

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Supplementary material 1

Parameters pH and aw of Bologna sausages elaborated with chia mucilage powder (MCP) and chia mucilage in gel (MCG).

Parameter	Days	FC[1]	F1	F2	F3	F4	F5	F6	SEM*
pH	0	$5.93^{d,D}$	$5.76^{e,D}$	$5.98^{c,C}$	$5.76^{e,C}$	$5.92^{d,C}$	$6.10^{a,B}$	$6.05^{b,B}$	0.012
	15	$6.06^{b,c,A}$	$6.05^{b,c,A}$	$6.10^{b,A}$	$6.02^{c,A}$	$6.01^{c,B}$	$6.14^{a,A}$	$6.10^{b,A}$	0.007
	30	$6.00^{b,c.C}$	$5.92^{d,C}$	$5.98^{c,d,B,C}$	$6.07^{a,b,A}$	$6.08^{a,A}$	$6.08^{a,C}$	$6.04^{a,b,c,B,C}$	0.009
	45	$6.03^{a,b,B}$	$5.99^{a,b,c,A,B}$	$5.99^{a,b,c,B,C}$	$6.04^{a,A}$	$5.99^{b,c,B}$	$6.00^{a,b,c,E}$	$5.95^{c,D}$	0.005
	60	$6.04^{a,A,B}$	$5.95^{b,B,C}$	$6.06^{a,A,B}$	$5.91^{b,B}$	$5.93^{b,C}$	$6.06^{a,D}$	$6.02^{a,C}$	0.007
aw	0	$0.975^{e,A}$	$0.980^{a,b,A}$	$0.980^{a,b,A,B}$	$0.977^{b,c,A}$	$0.976^{c,d,A}$	$0.981^{a,B}$	$0.980^{a,b,A,B}$	0.0003
	15	$0.974^{b,A}$	$0.976^{a,b,A,B}$	$0.978^{a,b,B,C}$	$0.977^{a,b,A}$	$0.978^{a,A}$	$0.975^{a,b,D}$	$0.978^{a,b,B,C}$	0.0003
	30	$0.974^{b,A}$	$0.975^{a,b,B}$	$0.976^{a,b,C}$	$0.976^{a,b,A}$	$0.976^{a,b,A}$	$0.979^{a,C}$	$0.975^{a,b,C}$	0.0003
	45	$0.976^{b,A}$	$0.979^{a,A}$	$0.977^{a,b,B,C}$	$0.977^{a,b,A}$	$0.975^{b,A}$	$0.977^{a,b,C,D}$	$0.980^{a,A,B}$	0.0003
	60	$0.975^{c,A}$	$0.978^{a,b,c,A}$	$0.982^{a,b,A}$	$0.978^{b,c,A}$	$0.972^{c,A}$	$0.985^{a,A}$	$0.983^{a,b,A}$	0.0006

*SEM- Standard error of the mean. ^{a,b,c,d,e}Mean values within the same line horizontally followed by the same line horizontally followed by the same lowercase letters did not show any significant difference ($P > 0.05$) by Tukey's test. ^{A,B,C,D,E}Mean values within the same column followed by the same capital letters did not show significant difference ($P>0.05$) by Tukey's test. [1] For treatments denominations, see Table 1.

CAPÍTULO 4

UNDERSTANDING THE ROLE OF CHIA (*SALVIA HISPANICA* L.) MUCILAGE ON OLIVE OIL-BASED EMULSION GELS AS A NEW FAT SUBSTITUTE IN EMULSIFIED MEAT PRODUCTS

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Artigo a ser submetido ao Food Hydrocolloids

Understanding the role of chia (*Salvia Hispanica* L.) mucilage on olive oil-based emulsion gels as a new fat substitute in emulsified meat products

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Abstract

The objective of this study was to evaluate the effect of chia mucilage (MC) as a gelling agent in olive oil-based emulsion gels (EGs) for use in emulsified meat products as a replacer of pork back fat. Six variables in combination with MC were evaluated using a Plackett-Burman design, as follows: alginate (ALG), collagen (COL), whey (WHEY), carboxymethylcellulose (CMC), transglutaminase (MTG), and carrageenan (CAR). After the first screening, a complete factorial design was applied to evaluate the interactions and properties of the selected variables (ALG, COL, and WHEY) for the manufacture of EGs. The rheological, technological, and microstructural properties of the emulsion gels were investigated. ALG and WHEY decreased the liquid release of the samples showing greater stability of EGs and meat model systems. The variable WHEY had a significant effect on the increase in luminosity of the EGs. The samples containing COL and ALG (run D) were unstable to heat treatment showing a high liquid release (12.58%). This study contributed to understanding the interactions among chia mucilage, biopolymers, and proteins, disclosing the potentiality of chia mucilage -based emulsion gels for the replacement of animal fat in emulsified meat products.

Keywords: emulsion gel, chia mucilage, meat emulsion, experimental design, olive oil, cold-set-gelation

1. Introduction

The high content of saturated fat in meat products, specially the emulsified products, has suggested a product reformulation aiming to help consumers to make healthier choices (Olmedilla-Alonso, Jiménez-Colmenero & Sánchez-Muniz, 2013; Jiménez-Colmenero, 2007). Several strategies have been proposed to reduce fat content and to improve the lipid profile of formulations containing pork back fat, the main source of saturated fat, by using vegetable oils rich in polyunsaturated fatty acids, dietary fibers, hydrocolloids and, recently, emulsion gels, hydrogels, and organogels. However, the use of these reformulations as single approach does not meet expected technological and sensorial properties since instability in the meat matrix during the processing stages and the negative effect in acceptance by consumers were observed (Herrero, Ruiz-Capillas, Pintado & Jiménez-Colmenero, 2018; Khalesi, Emadzadeh, Kadkhodaee & Fang, 2019; Pintado, Herrero, Jiménez-Colmenero,

Cavalheiro & Ruiz-Capillas, 2018; Freire, Cofrades, Pérez-Jiménez, Gomez-Estaca, Jiménez-Colmenero & Bou, 2018).

Thus, unraveling the interactions between different ingredients and the forms of addition to the formulations are crucial to produce stable and accepted meat products with fat reduction. Chia (*Salvia hispanica* L) seeds have the intrinsec ability to form a hydrogel, which is attribute to the mucilage mainly (Capitani, Corzo-Rios, Chel-Guerrero, Bentacur-Ancona, Nolasco & Tomas, 2015; Samateh, Pottackal, Manafirasi, Vidyasagar, Maldarelli, & John, 2018). Chia mucilage is comprised of soluble fibers including a tetramer of glucose, xylose, and glucuronic acid in the ratio 1: 2: 1 (Lin, Daniel & Whistler, 1994). A significant amount of uronic acids (glucuronic acid and galacturonic acid) and two other neutral sugars, namely arabinose and galactose, were also detected (Goh, Matia-Merino, Chiang, Quek, Soh & Lentle, 2016). This component (MC) has exceptional water holding capacity, as well as another important functional property as a thickener in the preparation of emulsions (Capitani, Nolasco & Tomas, 2016). Timilsena et al. (2016) evaluated the emulsifying properties of purified chia mucilage in terms of the emulsion activity index and the emulsion stability index. The surface activity and emulsifying properties of chia mucilage were found to be either superior or comparable to other common gums and industrial polysaccharides indicating the potential of mucilage as an effective thickener and stabilizer of processed foods. Another hypothesis is that mucilage of chia is an ingredient with many potentialities for use in emulsions gels, and consequently for reformulation of meat products. Besides, to this mucilage and chia have been attributed, health benefits, such as decrease of diabetes and obesity incidence, due to the delayed gastric emptying (Vázquez-Ovando, Rosado-Rubio, Chel-Cherrero & Betancur-Ancona, 2009; Capitani, Ixtaina, Nolasco & Tomas, 2013; Samateh et al. al., 2018).

Emulsion gels (EGs) are defined as emulsions with a gel-like lattice and solid mechanical properties as a soft matter formed by a network (Dickinson, 1989). In O/W emulsion gels, the colloidal structure may be related to a network of flocculated oil droplets or a network of crosslinked biopolymer molecules in the continuous phase among the droplets (Dickinson, 2013). Moreover, EGs have been applied in oil structuring improving the lipid profile, thus they used as an animal fat substitute in the meat products reformulation (Pintado et al., 2018).

These gelled lipid systems can be obtained by either thermal or non-thermal treatments, called as heat-set and cold-set gelation, respectively. The non-thermal gelation is more suitable to incorporate heat sensitive bioactive compounds (Tang, Chen & Foegeding,

2011), presenting other advantages such as the promotion of firmer texture, homogeneous structure, and greater water holding capacity (Mohammadian, Salami, Emam-Djomeh, Momen & Moosavi-Movahedi, 2018). Cold-set EGs can be elaborated with enzymes such as microbial transglutaminase (MTG), (Freire et al. 2018, Pintado et al., 2018), divalent salts including calcium chloride associated with gelling agents such as sodium alginate or whey protein (Khalesi et al., 2019), as well as the use of glucone delta lactone (GDL) for acidification (Tang et al., 2011). Most of these ingredients have been use to improve the technological properties of the EG mainly the texture profile.

The interactions between proteins and polysaccharides can result in attraction or repulsion, depending on the nature of the biopolymer and the continuous phase, leading to complexation or thermodynamic incompatibility, respectively (Tolstoguzov, 1995). Chia mucilage (MC) is a naturally occurring heteropolysaccharide considered safe for food use, with biocompatibility, offering opportunities for the food industry to use cleaner labels on their products (Elboutachfaiti, Delattre, Quéro, Roulard, Duchêne, Mesnard, & Petit, 2017). However, few studies have evaluated the use of MC combined with other biopolymers to create structures that mimic fat, aimed at create healthier alternatives for the reformulation of meat products (Capitani et al., 2016).

The behavior of MC interacting with other compounds in emulsions gels has not yet been investigated in other studies and can provide important information on the development of new fat substitutes. Therefore, this study aimed to develop a chia mucilage-based emulsion gel with olive oil, together with six gelling agents (sodium alginate, collagen, whey protein, carboxymethylcellulose, transglutaminase, and carrageenan) to replace pork back fat in emulsified meat products. The effects of six gelling agents combined with MC on both the EG and meat model systems were evaluated using a Plackett-Burman factorial design. Subsequently, the interactions and properties of the selected gelling agents (sodium alginate, collagen, and whey) were evaluated using a sequential factorial design, and the rheological, technological, and microstructural properties of the EGs were investigated.

2. Materials and methods

2.1 Materials

Chia seeds (*Salvia hispanica* L.) were purchased by Cereal Prime (Cereal Prime, São Paulo, Brazil). Carboxymethylcellulose (Nutrassim Food Ingredients, Extrema, Brazil), carrageenan (GENUGEL® MB-530 F, CP Kelco, Limeira, Brazil), whey protein (90% purity, Alibra Ingredients, Campinas, Brazil), collagen (Novaprom, Guaiçara, Brazil), sodium acid pyrophosphate (ICL Brasil, São Paulo, Brazil), calcium sulfate (Dinâmica Química Contemporânea Ltda, Indaiatuba, Brazil), sodium tripolyphosfate, sodium erythorbate, sodium nitrite and sodium alginate (Kerry do Brasil, Campinas, Brazil) were donated by respective food companies. Nile red dye was purchased from Sigma (Sigma–Aldrich Corporation St. Louis, USA). The microbial transglutaminase (MTG) Activa TG-S-NF was provided by Ajinomoto® with approximately 100 U/g of powder (Ajinomoto, São Paulo, Brazil). Extra virgin olive oil (D'Aguirre, Argentina) with 20.68% saturated fatty acids (SFA), 59.61% monounsaturated fatty acids (MUFA) and 19.71 polyunsaturated fatty acids (PUFA), was donated by Sandéleh Alimentos (Sorocaba, Brazil). Pork (*M. longissimus dorsi*) and pork back fat were obtained from a local market in Campinas (Brazil). After cleaning to remove apparent fat and aponeuroses, meat and pork fat were ground in a 5.0 mm disks and then frozen (-18 ºC) until further use.

2.2 Extraction of chia (Salvia hispanica L.) mucilage

Chia mucilage (MC) was obtained according to the procedure described by Coorey, Tjoe & Jayasena (2014) and Felisberto, Wahanik, Gomes-Ruffi, Clerici, Chang & Steel (2015) with some modifications. Whole chia seeds were soaked in distilled water (1:25 seed/water volume ratio) for 3 h at 60 °C using an electric cooker (STM, São Paulo, Brazil). The extracted mucilage was separated from the chia seeds using a 35/ CM-876 finisher depulper with a stainless-steel wire mesh of aperture size 0.26 mm (FMC do Brasil Indústria e Comércio Ltda, Araraquara, Brazil). The aqueous suspension obtained was subjected to drying in an LP 820 freeze-drier (São Paulo, Brazil) and stored in hermetically sealed metalized packaging, protected from moisture at room temperature.

2.3 Experimental design

2.3.1 Screening significant parameters by the Plackett–Burman design

In this study, a Plackett-Burman (PB) two-level factorial design with 15 experiments (Fig. 1) was used to evaluate the most significant variables in the preparation of the emulsion gels with chia mucilage (MC) and olive oil, with better technological performance to replace animal fat in emulsified meat products. In the PB design, each factor is considered independent and can be described as a first-order model:

$$Y = \beta_0 + \Sigma \beta i + x_i \quad (1)$$

where Y is the predicted response (pH, color parameters, rheological parameters, emulsion stability, and texture parameters), β_n is the regression coefficients, and x_i is the independent variable (carboxymethylcellulose (CMC, 0-1%), transglutaminase (MTG, 0-1.2%), collagen (COL, 0-2%), carrageenan (CAR, 0-0.8%), (ALG, 0-2%) and whey protein (WHEY, 0-3%). An alginate-based gelling agent was used, formulated with 0.75% sodium alginate, 0.75% calcium sulfate and 0.5% sodium acid pyrophosphate. The responses of the PB factorial design are presented in Table 1 and 2, for the evaluations of the emulsion gels and the meat emulsions, respectively. Twelve experiments and six independent variables were analyzed using Minitab® software 18.1 (Pennsylvania, USA), with each parameter at two levels: high (+1) and low or absent (-1), in addition to three repetitions at the central point. The responses were obtained after 24 h of elaboration of the emulsion gels.

Run	Variables (%)					
	CMC	MTG	COL	CAR	ALG	WHEY
1	1 (1)	-1 (0)	1 (2)	-1 (0)	-1 (0)	-1 (0)
2	1 (1)	1 (1.2)	-1 (0)	1 (0.8)	-1 (0)	-1 (0)
3	-1 (0)	1 (1.2)	1 (2)	-1 (0)	1 (2)	-1 (0)
4	1 (1)	-1 (0)	1 (2)	1 (0.8)	-1 (0)	1 (3)
5	1 (1)	1 (1.2)	-1 (0)	1 (0.8)	1 (2)	-1 (0)
6	1 (1)	1 (1.2)	1 (2)	-1 (0)	1 (2)	1 (3)
7	-1 (0)	1 (1.2)	1 (2)	1 (0.8)	-1 (0)	1 (3)
8	-1 (0)	-1 (0)	1 (2)	1 (0.8)	1 (2)	-1 (0)
9	-1 (0)	-1 (0)	-1 (0)	1 (0.8)	1 (2)	1 (3)
10	1 (1)	-1 (0)	-1 (0)	-1 (0)	1 (2)	1 (3)
11	-1 (0)	1 (1.2)	-1 (0)	-1 (0)	-1 (0)	1 (3)
12	-1 (0)	-1 (0)	-1 (0)	-1 (0)	-1 (0)	-1 (0)
13-15 (Central points)	0 (0.5)	0 (0.6)	0 (1)	0 (0.4)	0 (1)	0 (1.5)

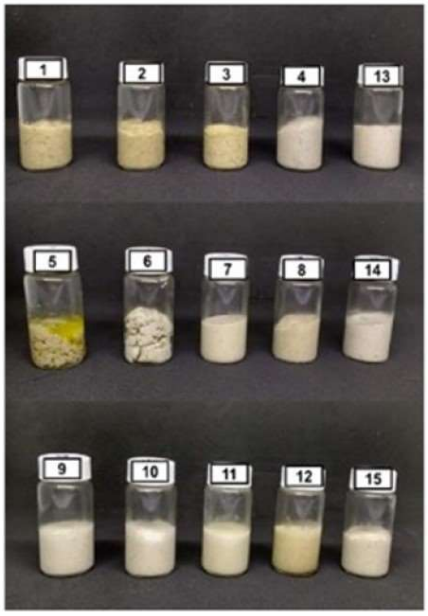

Fig 1. Plackett-Burman factorial design (left) (experimental real values are in parenthesis) and appearance of emulsion gels (right) (all with 5% of chia mucilage and 40% of olive oil). CMC: carboxymethylcellulose; MTG: transglutaminase; COL: collagen; CAR: carrageenan; ALG: alginate (0.75% sodium alginate, 0.75% calcium sulfate and 0.5% sodium pyrophosphate); WHEY: whey protein isolate.

Table 1. Responses (pH values, color, emulsion stability, rheological and texture parameters) of Plackett-Burman factorial design of emulsion gels.

Run	pH	Color			Emulsion stability (%)		Rheological		Texture
		L*	a*	b*	Liquid released	Fat released	G' (Pa)[1]	G" (Pa)[1]	Force (N) Penetration
1 (CMC, COL)	5.64	64.12	3.67	20.19	18.31	18.29	8236.67	2730.00	0.26
2 (CMC, MTG, CAR)	6.54	61.92	4.60	22.04	0.58	0.57	3783.33	1503.33	0.20
3 (MTG, COL, ALG)	5.92	63.76	4.21	21.17	0.44	0.13	8476.67	1548.33	0.48
4 (CMC, COL, CAR, WHEY)	6.10	75.35	2.06	13.50	0.00	0.00	8900.00	2040.00	0.48
5 (CMC, MTG, CAR, ALG)	6.60	NEG[2]	NEG[2]	NEG[2]	NEG[2]	NEG[2]	NEG[2]	NEG[2]	NEG[2]
6 (CMC, MTG, COL, ALG, WHEY)	5.59	75.99	2.26	13.65	0.00	0.00	14800.00	2540.00	1.27
7 (MTG, COL, CAR, WHEY)	6.27	72.71	2.59	15.35	0.00	0.00	3126.67	609.00	0.15
8 (COL, CAR, ALG)	6.30	64.80	3.30	17.89	34.09	34.01	13550.00	2940.00	0.71
9 (CAR, ALG, WHEY)	6.06	73.74	2.24	14.38	0.00	0.00	3120.00	840.50	0.14
10 (CMC, ALG, WHEY)	5.80	75.85	2.03	13.54	0.00	0.00	5295.00	1655.00	0.36
11 (MTG, WHEY)	5.90	73.06	2.84	17.31	0.00	0.00	116.00	46.10	0.00[3]
12 (-----)	5.58	62.15	4.52	22.20	4.66	0.08	461.50	180.50	0.00[3]
13-15 (Central points)	5.92 ± 0.13	75.22 ± 0.28	2.27 ± 0.10	14.08 ± 0.38	0 ± 0.00	0 ± 0.00	4201.67 ± 265	1110 ± 86	0.31 ± 0.02

[1] Frequency at 1 Hz and fixed strain within the linear viscoelastic domain (0.1%). [2] NEG: No emulsion gel formation. [3] Fluid gels, below the detection limit of the method. See Fig 1 for a description of runs and variables.

Table 2. Responses (pH values, emulsion stability and texture parameters) of Plackett-Burman factorial design of meat emulsion evaluations elaborated with different emulsions gels describe in the Fig 1.

Run	Emulsion stability (%)			Texture		
	pH	Liquid released	Fat released	Hardness (N)	Chewiness	Elasticity
1 (CMC, COL)	6.03	10.99	0.62	7.20	5.42	0.903
2 (CMC, MTG, CAR)	6.06	10.61	0.58	8.91	6.89	0.913
3 (MTG, COL, ALG)	5.98	9.60	0.50	7.99	6.60	0.927
4 (CMC, COL, CAR, WHEY)	5.94	3.39	0.22	6.39	4.63	0.897
5 (CMC, MTG, CAR, ALG)	5.86	5.55	0.34	5.99	4.32	0.901
6 (CMC, MTG, COL, ALG, WHEY)	5.84	7.27	0.49	6.92	5.11	0.892
7 (MTG, COL, CAR, WHEY)	5.79	10.66	0.68	8.14	6.25	0.909
8 (COL, CAR, ALG)	5.74	11.59	0.76	8.21	5.84	0.898
9 (CAR, ALG, WHEY)	5.92	8.71	0.65	11.30	8.01	0.902
10 (CMC, ALG, WHEY)	5.82	6.22	0.48	8.77	5.86	0.891
11 (MTG, WHEY)	5.86	18.80	1.31	10.53	7.93	0.903
12 (-----)	5.89	15.42	0.94	8.50	6.17	0.901
13-15 (Central points)	5.87 ± 0.06	9.67 ± 1.02	0.62 ± 0.11	8.56 ± 0.46	6.35 ± 0.58	0.907 ± 0.00

2.3.2 Complete Factorial design

In base or the more adequate technological characteristics a 2^3 complete factorial design with two levels was performed after the selection of factors by the PB design, totaling 11 experiments, including 3 replicates at the central point (Fig. 2). The three variables evaluated were collagen (COL, 0-2%), alginate (ALG, 0-2%) and whey protein (WHEY, 0-3%). The responses of the complete factorial design are presented in Table 3, for the evaluations of the emulsion gels (pH, color, emulsion stability, rheological parameters, and texture) and the meat emulsions (emulsion stability and pH).

Run	Variables (%)		
	COL	ALG	WHEY
A	-1 (0)	-1 (0)	-1 (0)
B	1 (2)	-1 (0)	-1 (0)
C	-1 (0)	1 (2)	-1 (0)
D	1 (2)	1 (2)	-1 (0)
E	-1 (0)	-1 (0)	1 (3)
F	1 (2)	-1 (0)	1 (3)
G	-1 (0)	1 (2)	1 (3)
H	1 (2)	1 (2)	1 (3)
I, J, K (Central points)	0 (1)	0 (1)	0 (1.5)

Fig 2. Complete factorial design (left) (experimental real values are in parenthesis) and appearance of emulsion gels (right) (all with 5% of chia mucilage and 40% of olive oil). COL: collagen; ALG: alginate (0.75% sodium alginate, 0.75% calcium sulfate and 0.5% sodium pyrophosphate); WHEY: whey protein isolate.

Table 3. Responses of complete factorial design of emulsion gels (pH values, color and emulsion stability parameters) and meat emulsion (emulsion stability parameters, pH values) elaborated with different emulsions gels describe in the Fig 2.

Run	Emulsion gel									Meat emulsion		
		Color			Emulsion stability (%)		Rheological		Texture	Emulsion stability (%)		
	pH	L*	a*	b*	Liquid released	Fat released	G' (Pa)[1]	G'' (Pa)[1]	Force (N) Penetration	Liquid released	Fat released	pH
A (-----)	5.76	59.65	4.72	22.92	8.01	4.90	317.00	118.00	0.00	9.56	0.61	6.07
B (COL)	5.82	63.81	3.76	19.87	1.75	0.51	7990.00	1860.00	0.75	7.93	0.56	6.01
C (ALG)	5.98	65.04	4.28	21.75	0.32	0.08	3716.67	878.00	0.34	5.03	0.32	5.87
D (COL, ALG)	5.96	65.98	3.53	20.49	12.58	12.56	13000.00	2920.00	0.73	4.69	0.29	5.88
E (WHEY)	5.71	72.85	2.88	17.73	1.67	0.11	60.90	35.23	0.00	3.18	0.24	5.99
F (COL, WHEY)	5.75	75.95	2.48	15.20	0.12	0.00	3483.33	673.33	0.29	3.77	0.23	5.99
G (ALG, WHEY)	5.86	77.66	2.37	15.40	0.00	0.00	2173.33	522.67	0.14	1.40	0.17	5.98
H (COL, ALG, WHEY)	5.80	76.61	2.37	15.53	0.00	0.00	8530.00	1583.33	0.49	3.65	0.26	6.05
I, J, K (Central points)	5.99 ± 0.02	75.98 ± 0.89	2.22 ± 0.12	14.93 ± 0.42	0.00 ± 0.00	0.00 ± 0.00	1782.22 ± 160.57	491.89 ± 46.13	0.121 ± 0.01	3.02 ± 0.55	0.183 ± 0.08	6.00 ± 0.02

[1] Frequency at 1 Hz and fixed strain within the linear viscoelastic domain (0.1%). See Fig 2 for a description of runs and variables.

2.4 Chia mucilage-based emulsion gels preparation

Emulsion gels (EGs) were prepared with 40% olive oil and 5% (as a fixed level) of chia mucilage together with various gelling agents, according to the Plackett-Burman and Factorial designs (Fig. 1 and Fig. 2, respectively). The percentages of olive oil, chia mucilage, and gelling agents were defined in preliminary tests. Six ingredients were evaluated as gelling/stabilizing agents, as follows: carboxymethylcellulose (CMC), transglutaminase (MTG), collagen (COL), carrageenan (CAR), alginate (ALG) (0.75% sodium alginate, 0.75 % calcium sulfate, and 0.5% sodium acid pyrophosphate) and whey protein (WHEY) (Herrero et al., 2018). The gelling agents collagen and carrageenan were previously dissolved in water at 40 °C and the whey protein was dissolved in water at 70 °C, and cooled to room temperature and then added for homogenization with the remainder of the ingredients. The emulsion gels were prepared according to the procedure described by Pintado et al. (2015) with some modifications. Initially, chia mucilage (MC) was hydrated with part of the water of the formulation until it formed a paste, which was then homogenized (Thermomix TM5, Vorwerk, Wuppertal, Germany) with water for 90 seconds at 1,100 rpm. After hydration of the mucilage, the gelling agents were added (according to the experimental designs, Fig. 1 and Fig. 2) and the homogenization was continued for one minute at 4,000 rpm. Olive oil was gradually incorporated into the mixture for four minutes at 3,500 rpm, and then for one minute at 5,100 rpm. The emulsion gels were placed in polystyrene containers and stored in a refrigerated chamber at 5 °C for 24 hours before analyses.

2.5 Preparation of meat emulsion model systems with chia mucilage-based emulsion gels

Meat emulsion model systems were prepared to evaluate the interactions between the emulsion gels and a traditional emulsified meat product formulation (60% pork; 20% pork fat or emulsion gel -in experiments with EG, the animal fat was completely removed- ,1.5% NaCl; 0.25% sodium tripolyphosphate; 0.015% sodium nitrite; 0.05% sodium erythorbate; and 18.18% ice).

2.6 pH determination

The pH of emulsion gels and meat model system was evaluated using a MA 130 Mettler pH meter using a penetration probe at different places of the sample. The analysis was conducted in triplicate.

2.7 Color determination

Emulsion Gels (EGs) were subjected to color analysis using a Colorquest II spectrophotometer (Hunter-Lab, USA) considering the CIELAB color system. Measurements were taken in six repetitions per emulsion gel.

2.8. Penetration test

Penetration tests in the EGs were performed using a TA-XT Plus texture analyzer (Stable Microsystems Ltd., Surrey, UK) according to Herrero et al. (2011) with some modifications. The emulsion gels were placed in cylindrical flasks (6x3 cm) and centrifuged for one minute at 2000 rpm to eliminate air bubbles. The samples were kept for 24 h at refrigeration temperature (5 °C). The analyses were performed with a cylindrical stainless-steel probe of 6 mm diameter inserted into the sample at a distance of 10 mm and speed of 0.8 mm/s. The penetration force (N) was derived from the force deformation curves. The measurements were carried out in six replicates.

2.9 Texture Profile Analysis (TPA)

The TPA was performed at room temperature in meat model system (PB design) using a texture analyzer TA-XT2i (Texture Technologies Corp., Scarsdale, NY). Ten cylinders with 20 mm diameter and 20 mm height were used for each experiment. The samples were compressed to 30% of its original weight, with speed test, pretest and post test of 1.00 mm/s and force of 0.10 N. A P-35 probe was used (long shaft/regular basis). The parameters evaluated were hardness (N), springiness and chewiness.

2.10 Emulsion stability

The emulsion stability of the different emulsion gels and meat model system was performed according to Hugues, Cofrades & Troy (1997) with some modifications. Approximately 20 g of emulsion gel was weighed in plastic containers and centrifuged for 1 min at 3600 rpm. Subsequently, the samples were heated at 70 ºC in a water bath for 30 min and then centrifuged again for 3 min at 3600 rpm. The supernatant obtained after centrifugation of the samples was placed in 50 mL weighed beakers that were dried at 100 ºC for approximately 14 h. The total volume of liquid (% TEF) and percent fat were calculated. Five measurements per sample were performed. The volumes of total expressible fluid (TEF) and the percentage fat were calculated as follows:

TEF = (weight of beaker with the liquid released) – (weight of the empty beaker)
% TEF = TEF/sample weight x 100
% Fat = (weight of beaker + lipids after drying) – (weight of the empty beaker)/ sample weight x 100

2.11 Oscillatory rheological measurements

The rheological properties of the emulsion gels were measured according to Paglarini, Furtado, Biachi, Vidal, Martini, Forte, Cunha & Pollonio (2018) with some modifications, using a Physica MCR301 rheometer (Anton Paar, Austria) with stainless steel cone-plate geometry (50 mm, truncation 208 μm, and 2° angle). Previously, a strain sweep (0.01-100%) was performed to identify the linear viscoelasticity region (LVR) at a fixed frequency of 1 Hz. Then, a frequency sweep (0.1-10 Hz) was performed with the strain set within LVR. Dynamic mechanical behavior was obtained by recording the storage modulus (*G'*) and the loss modulus (*G''*) as a function of frequency at 5 ºC. The measurements were performed in triplicate.

2.12 Fluorescence microscopy analysis

The microstructure of emulsions gels selected based on the best properties of texture, stability and color was evaluated in a Carl Zeiss Axio Scope A1 microscope (Zeiss, Oberkochen, Germany) and Zen software 2.3. The FS43HE fluorescence filter (emission: 573 nm and excitation: 554 nm) was used for the dye Nile red with objective lens of 40x. For that, a small amount of dye was added to the gel, which was placed gently on a glass slide and covered with a coverslip. Representative images were collected at different places of the slide.

2.13 Statistical analysis

Both PB and complete factorial designs were performed using Minitab® 18.1 software (Pennsylvania, USA) at a 95% confidence level ($p < 0.05$) for the selection of the three variables and evaluation of effects and interactions in the factorial design. All data were analyzed by analysis of variance (ANOVA) to determine the significant effect of each variable, as well as their interactions in the responses. The statistical parameters included the coefficient of determination (R^2), F test and lack of fit test.

3. Results and discussion

3.1 Screening experimental factors

The EGs (emulsion gels) technological responses pH, objective color, emulsion stability, rheological and texture parameters were evaluated aiming at using the emulsion gels as fat substitutes to improve the nutritional profile of meat products.

3.1.1 pH and color measurements

The pH values of the emulsion gels ranged from 5.58 (run 12, only chia mucilage and olive oil, without gelling agents) to 6.60 (run 5, combination of CMC, MTG, CAR and ALG), as shown in Table 1. Table 4 shows the main effects of the six variables (CMC, MTG, COL, CAR, ALG and WHEY) on the responses (at a confidence level of 95%). A significant positive effect was observed for the variables carrageenan (CAR) and transglutaminase (MTG), individually, since PB does not allow to evaluate the interactions. According to the manufacturer, the carrageenan used in this study (GENUGEL® MB-530F) has pH values between 8 and 10, and contains potassium chloride in its commercial composition, which may explain the higher pH values observed. The transglutaminase, at the concentrations used in this study (0-1.2%), may have increased the pH value of the emulsion gels due to eventual ammonia release from the catalytic reactions to form covalent bonds between glutamine and lysine residues of proteins or deamidation of glutamine residues (Ohtsuka, Umezawa, Nio & Kubota, 2001). However, the other four variables (CMC, COL, ALG and WHEY) did not lead to any significant effects on the pH values, which ranged from 5.6 to 5.8. In view of pH values range observed, all variables are suitable for application as pork back fat substitutes without compromising the pH values in the final product.

The color is one of the most important criteria used by consumers in their purchase decisions (McClements, 2016). The color of the emulsion gels is shown in Fig 1. Emulsion gels addition may influence the color attributes of the product, depending on the percentages used in the formulations of meat products (Paglarini et al., 2018). As can be seen in Table 4, there was a significant effect of the variable WHEY on the L * parameter, with very high luminosity values.

The dispersion characteristics of an emulsion are strongly influenced by the concentration of droplets (McClements, 2002; Chanamai & McClements, 2001). In a more concentrated emulsion, a significant fraction of the incident light can propagate back to the surface, and the dispersion phase is largely responsible for the emulsion lightness (McClements, 2016). Possibly, the emulsion gels

made with WHEY were more stable, with a larger number of droplets with smaller size (as shown in Fig 3).

The increase in L* values due to the of WHEY can have a positive impact on the EGs, since MC has a brown coloration, which can negatively affect the consumers' acceptance, and a higher L* value would help to decrease the undesirable brownish coloration. The brown appearance of MC is related to the extraction process, in which the seeds were subjected to a strong pressure at the moment of seed separation and the mucilage and part of the outer covering of the seed may have incorporate into the MC. Regarding the color parameter a*, which is correlated to the red tonality, significant negative effects of the variables COL, ALG, and WHEY (Table 4) were observed. On the other hand, the variable MTG resulted in positive effects both in a* and b* values (referring to yellow color). Pintado, Ruiz-Capillas, Jiménez-Colmenero, Carmona & Herrero (2015) studied emulsion gels containing chia flour, olive oil, and gelling agents (transglutaminase, alginate, or gelatin), and observed higher b* and lower a* values for the treatments with transglutaminase and gelatin, respectively, which is similar to the findings of this study.

Table 4. Effects of independent variables (CMC, MTG, COL, CAR, ALG and WHEY) on the responses in Plackett-Burman factorial design.

	Emulsion gel								Meat emulsion added of emulsion gel	
	pH	L*	a*	b*	Liquid released	Force Penetration test	G' [1]	G'' [1]	Liquid released	Hardness
Model	**(<0.01)**	**(<0.01)**	**(<0.01)**	**(<0.01)**	(0.27)	(0.05)	**(0.01)**	**(<0.01)**	**(0.04)**	(0.15)
CMC	0.04 (0.63)	-0.90 (0.62)	-0.09 (0.39)	-0.31 (0.56)	0.50 (0.93)	0.25 (0.07)	2816 (0.06)	**1015 (<0.01)**	**-5.12 (0.01)**	**-1.75 (0.03)**
MTG	**0.22 (0.02)**	-2.82 (0.15)	**0.53 (<0.01)**	**1.89 (<0.01)**	-5.45 (0.35)	0.09 (0.46)	-755 (0.56)	-392 (0.10)	1.03 (0.47)	-0.32 (0.63)
COL	-0.11 (0.20)	3.07 (0.12)	**-0.44 (<0.01)**	**-1.88 (<0.01)**	4.05 (0.48)	**0.37 (0.02)**	**6597 (<0.01)**	**1066 (<0.01)**	-1.97 (0.18)	**-1.52 (0.047)**
CAR	**0.57 (<0.01)**	-2.47 (0.20)	-0.04 (0.69)	-0.23 (0.66)	5.76 (0.33)	-0.04 (0.72)	-29 (0.98)	170 (0.44)	-2.96 (0.06)	-0.17 (0.80)
ALG	0.04 (0.62)	-0.59 (0.75)	**-0.29 (0.03)**	-1.07 (0.07)	5.71 (0.33)	**0.38 (0.02)**	**4225 (0.01)**	**700 (0.01)**	**-3.49 (0.04)**	-0.08 (0.90)
WHEY	-0.14 (0.11)	**13.06 (<0.01)**	**-1.79 (<0.01)**	**-6.55 (<0.01)**	**-13.57(0.04)**	0.06 (0.63)	-647 (0.62)	**-493 (0.049)**	-1.45 (0.31)	0.87 (0.21)
R^2	90.84%	91.78%	98.35%	97.21%	61.62%	78.92%	87.43%	91.45%	81.31%	69.44%

[1] Frequency at 1 Hz and fixed strain within the linear viscoelastic domain (0.1%). Bold values are statistically significant ($p < 0.05$), in parenthesis p value. Only the responses that had some significant variable are described in the table. See Fig 1 for a description of runs and variables.

3.1.2 Stability of emulsion gels

Regarding the stability of the emulsion gels, it is worth mentioning the positive performance of MC, which was present in all formulations. Mucilage has a hydrophilic structure that provides the ability to increase the viscosity of the aqueous phase, thereby reducing the mobility of the oil droplets, preventing coalescence (Avila-de la Rosa, Álvarez-Ramírez, Vernon-Carter, Carrillo-Navas & Pérez-Alonso, 2015; Garti & Leser, 2001). It is reported that MC has the intrinsic ability to form hydrogels concomitant with moisture retention (Samateh et al., 2018). The results of run 12 (Table 1) show a low liquid release, which represents a good performance of the chia mucilage as an emulsifier agent since there was no other variable rather than MC in this run. The high viscosity and higher water retention of MC is related with its content of 4-O-methyl glucuronic acid that forms intermolecular bonds in aqueous medium (Timilsena et al., 2016).

On the other hand, a higher liquid release observed in runs 1 (CMC and COL) and 8 (COL, CAR, ALG), with 18.31% and 34.09%, respectively (Table 1), suggesting the instability of the emulsion gels. Different behaviors could be observed in EGs depending on each biopolymer matrix, since the incorporated oil droplets may or may not interact with the gel matrix. The oil droplets that can interact with the gel matrix are called active fillers. Conversely, there are also the inactive fillers, which have no chemical or physical affinity with the molecules of the gel matrix. In this case, when there is no affinity, as observed in runs 1 and 8, the polysaccharides do not completely cover the oil droplets inhibiting the formation of an adjusted or concise polymer matrix, resulting in weak interactions (Abhyankar, Mulvihill & Auty, 2011; Kim, Gohtani, Matsuno, & Yamano, 1999).

With regard to the run 5, containing CMC, MTG, CAR, and ALG (Table 1 and Fig. 1), no gel formation was observed, with the coalescence of the fat globules due to thermodynamic incompatibility phenomenon. According to Tolstoguzov (1995), the interactions between proteins and polysaccharides can be either attractive or repulsive, resulting in thermodynamic aggregation or incompatibility, depending on the nature of the biopolymers and the conditions of the continuous phase, respectively, which is a phenomenon widely observed for mixtures of proteins and polysaccharides, occurring mainly due to the low entropy of the mixture (Polyakov, Grinberg & Tolstoguzov, 1997). In addition, phase separation may have been influenced by competition between the biopolymers by water for gelation.

A significant negative effect (Table 4) of the variable WHEY was observed for the response liquid release in the emulsion gels runs. Whey proteins are widely used in the elaboration of emulsion gels, especially due to their high water-binding capacity, thickening, emulsifying and gelling properties, nutritional characteristics, and wide availability as a by-product from the dairy industry (Khalesi et al. al., 2019; Dickinson, 2012). According to Ye & Singh (2006), whey proteins,

when adsorbed on the surface of the emulsion droplet, create an electrostatic barrier against flocculation and coalescence by stabilizing the system.

3.1.3 Texture property (force penetration) and rheological parameters of emulsion gels

According to Dickinson (2012), one of the main consequences of the presence of additional gelling agents (such as those used in this work) is the change in the nature of the interactions between the droplet surface and the gel matrix material and therefore of rheological properties. In rheological measurements, the parameters G' and G'' indicate the solid-like and liquid-like behavior, respectively. For gels, typically the elastic modulus (G') dominates over the viscous modulus (G'') at small oscillation stresses. All samples, apart from run 5, showed a typical gel-like behavior ($G' > G''$) and a frequency independent behavior (Table 1). Even the formulation with only mucilage (run 12) showed a dominant G' (Table 1) forming a gel-like structure, as also reported by García-Salcedo, Torres-Vargas, Real, Contreras-Jiménez & Rodríguez-García (2018). Table 4 shows the significant positive effects of the COL and ALG variables for the penetration force, G’ and G" responses in the evaluation of chia mucilage-based emulsions. The highest values found for penetration force (Table 1) were in the runs 6 (CMC, MTG, COL, ALG and WHEY) and 8 (COL, CAR and ALG), which had COL and ALG in the formulation. For the chia mucilage-based gel emulsion, there is a correlation between the responses of penetration force and G', since both demonstrate the mechanical properties of stronger gels (runs 6 and 8).

The collagen powder used in this study, due to the presence of shorter protein chains and the higher exposure of hydrophilic groups, such as hydroxyproline residues, in combination with hydrophilic groups of chia mucilage, possibly favored interaction with water and, consequently, gelling properties (Máximo & Cunha, 2010). Oliver, Scholten & Aken (2015) observed only a slight increase in fracture stress in their study with gelatin and casein in emulsion filled-gels gels when modified the type of fat (saturated solid fat and sunflower oil). The hypothesis suggested by the authors is that because of the polymeric nature of the network in the gelatin gels (formed by helix chains) and high binding capacity between these chains, the fracture is rather difficult and as a consequence, the gelatin gels are quite elastic , which corroborates with the significant effects of collagen found for the elastic modulus G' in our study.

Sato, Moraes & Cunha (2014) found lower values of mechanical properties in gelled emulsions with pure alginate than emulsions with gelatin in their study of gelled emulsions produced at high pressure homogenization (0-60 MPa). The stability of the mixed gelled systems (alginate and gelatin) has been improved and was associated to the emulsifying properties of gelatin with the high pH resistance of alginate. In the present study, higher values of G' and penetration force were also

observed when collagen and alginate were present in the same run, suggesting a possible interaction, moreover which could be will evaluate in the next step of the complete factorial design. Pintado, Ruiz-Capillas, Jiménez-Colmenero, Carmona & Herrero (2015) also observed higher values of penetration force and gel strength in emulsion gels containing alginate or gelatin than to samples with transglutaminase in the development of emulsion gels containing chia (flour or seed) and cold gelling agents.

3.1.4 Effects of adding EGs on texture and stability of emulsified meat model systems

The responses of the PB factorial design for the evaluations of the meat emulsions (meat model systems) elaborated with EGs are presented in Table 2. Table 4 shows the effects of the variables on the responses of the meat emulsion evaluations with the EGs that had some significant variable (released liquid and hardness). Negative effects of CMC and ALG were observed on the parameter liquid release of the meat model system containing EGs, as shown in Table 4. According to Morin, Temelli, & McMullen, (2004), the variable CMC is a water-soluble anionic polymer that may interact with the meat proteins by crosslinking between its negatively charged carboxy groups with the positively charged side chains of the amino acids in the myofibrillar proteins. Gibis, Schuh, Allard & Weiss (2017) reported significant reductions in weight loss in meat model systems with the addition of 1 to 2% carboxymethylcellulose. Sodium alginate, which is hydrophilic in nature, reduced the liquid release in meat model systems probably due to the strong electrostatic interactions and hydrogen bonds between meat proteins, as observed by Yao, Zhou, Chen, Ma, Li & Chen (2018) in myosin gels made with the addition of sodium alginate of different molecular weights.

In addition, negative effects of CMC were also observed on the parameter firmness of the meat model systems made with EGs (Table 3). Several studies have shown a decrease in hardness in meat products containing 1% carboxymethylcellulose in the formulations (Gibis et al., 2017; Schuh, Allard, Herrmann, Gibis, Kohlus & Weiss, 2013; Han & Bertram, 2017). According to Schuh et al. (2013), the lower firmness may be due to the destabilization of the meat batter with the addition of CMC, since after cooking, the conversion to a cohesive protein network no longer occurs. It is possible that the addition of CMC to the meat system interferes with or disrupts the protein-water or protein-protein network, which in turn may decrease the gel strength (Han & Bertram, 2017).

The EGs with COL incorporated into the meat emulsions suffered the effects of ionic changes, heating and cooling, which are inherent to the meat products elaboration process, resulting in a lower firmness of the model systems evaluated (Table 2 and Table 4, responses and effects, respectively). Heating and subsequent cooling can lead to certain protein degradation and make collagen less effective in forming cross-links through the triple helix (Babin & Dickson, 2001). In

addition, collagen gels may undergo behavior changes due to ionic strength and pH (Neklyudov, 2003; Máximo & Cunha, 2010), as also observed in the present study in meat model systems.

Considering the technological application of MC as fat substitute in meat products and the greater complexity of its hydrophilic structure, its functional potential is remarkable, which may be even more effective when interacting with some of the evaluated polymers. Therefore, the variables WHEY, COL, and ALG were selected for optimization of EG runs in a complete factorial design, as described below.

3.2 Effects of the variables alginate, collagen, and whey on emulsion gels properties and meat model systems

The complete experimental design allowed to verify the interactions between the variables selected in the Plackett-Burman design (alginate, collagen, and whey) to produce EGs with technological and mechanical properties suitable for the proposed application. The matrix of the design and the appearance of the emulsion gels (EGs) are shown in Fig. 2. The responses of complete factorial design are presented in Table 3. Statistical analysis data including means of the analysis of variance (ANOVA), degrees of freedom, and F value are available in the Supplementary Material (S1).

The variables WHEY and ALG had significant effects on pH of the EGs (Table 5), with a negative effect observed for WHEY, due to its intrinsic lower pH value, once it is a co-product of cheese manufacturing (Smithers, 2008). In contrast, ALG exerted a positive effect on this response, with no significant effects on the final pH values of the meat model systems, thus the addition of ALG probably not interfere with the pH of emulsified meat product formulations.

As can be seen in Table 5, there was a significant effect of WHEY on the color parameters, with positive effects on the parameter L* and negative effects on the color coordinates a* and b*. According to Figure 2, a clearer appearance was observed for all whey-based formulations, which corroborates with the other results discussed in the PB design.

Similar to that observed in the PB design (Table 1), a relationship was observed between the penetration force and the rheological properties (higher penetration force, G' and G'') for the formulations with the addition of ALG or COL (Table 3). The combination COL + ALG (concentration of 2% each, run D) showed high viscous and elastic modulus values and a high liquid release. The time-dependent behavior of small deformations revealed stable emulsions (viscous and elastic modulus), from the rheological point of view, despite the temperature-sensitive gel network, evaluated in the emulsion stability test (Section 2.10), as shown by the high liquid release (Table 3). The positive effect of the combination COL + ALG + WHEY on the penetration force (Table 5) is

probably due to the total increase in the components of the system (7% w/w) rather than some possible interaction.

The variable WHEY exhibited a negative contribution to the parameter's penetration force and G' and G'' modulus. In the native form, whey proteins have a compact and rigid structure stabilized by intramolecular interactions, such as hydrophobic, electrostatic, hydrogen bonding, and disulfide interactions (Kiokias, Dimakou & Oreopoulou, 2007). As cold-set gelation was used, probably there was no effective unfolding of proteins, thus impairing its gelation property.

Table 5. Effects of three independent variables (COL, ALG and WHEY) on the responses and their interactions in complete factorial design.

	Emulsion gel							Meat emulsion
	pH	L*	a*	b*	Force Penetration test	G' [1]	G" [1]	Liquid released
Model	**(0.03)**	**(0.02)**	**(0.01)**	**(0.02)**	**(<0.01)**	**(<0.01)**	**(<0.01)**	**(0.04)**
COL	0.01 (0.74)	1.79 (0.13)	**-0.53 (0.03)**	**-1.67 (0.03)**	**0.45 (0.00)**	**6683.9 (0.00)**	**1370.7 (<0.01)**	0.22 (0.63)
ALG	**0.14 (0.01)**	**3.26 (0.04)**	-0.33 (0.06)	-0.64 (0.17)	**0.17 (<0.01)**	**3892.2 (<0.01)**	**804.4 (<0.01)**	**-2.42 (0.03)**
WHEY	**-0.10 (0.03)**	**12.15 (<0.01)**	**-1.55 (<0.01)**	**-5.29 (<0.01)**	**-0.22 (<0.01)**	**-2694 (<0.01)**	**-740.4 (<0.01)**	**-3.80 (0.01)**
COL*ALG	-0.05 (0.10)	-1.84 (0.12)	0.15 (0.22)	1.11 (0.07)	-0.08 (0.06)	**1136.1 (0.01)**	**180.6 (0.03)**	0.74 (0.2)
COL*WHEY	-0.02 (0.43)	-0.76 (0.39)	0.33 (0.06)	0.48 (0.25)	**-0.12 (<0.01)**	**-1794.3 (<0.01)**	**-521.3 (<0.01)**	1.21 (0.09)
ALG*WHEY	-0.04 (0.12)	-0.52 (0.54)	0.01 (0.90)	-0.37 (0.35)	0.01 (0.57)	-312.6 (0.11)	-105.6 (0.08)	1.47 (0.06)
COL*ALG*WHEY	-0.01 (0.78)	-0.24 (0.77)	0.05 (0.64)	0.22 (0.54)	**0.11 (<0.01)**	331 (0.1)	30.6 (0.45)	0.09 (0.84)
R^2	99.17%	99.53%	99.65%	99.63%	99.96%	99.97%	99.94%	98.94%

[1] Frequency at 1 Hz and fixed strain within the linear viscoelastic domain (0.1%). Bold values are statistically significant ($p < 0.05$), in parenthesis p value. Only the responses that had some significant variable are described in the table. See Fig 2 for a description of runs and variables.

However, the heating process during the emulsion stability tests led to remarkable positive stability results (for EG and the meat emulsion).

Regarding the liquid release of EGs, it was not possible to obtain a model through the factorial design to estimate the effects, once the standard error was equivalent to zero. However, the results of emulsion stability are shown in Table 3. As discussed in the PB design, strong and stable interactions are observed between WHEY, MC, and olive oil, which was also shown by the second design, since a small or no liquid release was observed in all experiments with the addition of WHEY, therefore the effects were not measured. In contrast, the result of run D, with the addition of COL and ALG, which exhibited a high liquid release (12.58%) demonstrated the instability of this run. It is possible that the combination COL + ALG resulted in weak interactions with little chemical or physical affinity between the molecules that make up EG, besides being more unstable to the heat treatment (Babin & Dickson, 2001; Abhyankar et al., 2011).

A lower liquid release was observed in meat model systems with EGs containing ALG or WHEY (Table 3), probably due to the ability of alginate to increase the binding forces between the myosin-alginate systems (Yao et al., 2018). In relation to WHEY, it is worth emphasizing that heating leads to a greater volume and flexibility of the molecule and increases its functionality as an emulsifier (Raikos, 2010). However, no significant interaction of ALG and WHEY was observed for the meat emulsion stability (Table 5), despite a low liquid release was observed (Table 3) in the run G, which was close to the values found for the control formulation (1.40 and 1.03, for run G and control, respectively).

3.3 Fluorescence microscopy and characteristics of the EGs fat globules

The microstructure of the emulsion gels was evaluated by fluorescence microscopy showed in the Figure 3). The microscopic images of EGs clearly demonstrate the impact of the different gelling agents used. With respect to the EG made with collagen COL (Fig. 3A), it is possible to observe larger, more dispersed oil droplets with some flocculation and interfacial layers weakly incorporated into the gel network, represented by the image showing an undefined contour around the droplets, thus representing lower emulsion stability, which can be directly related to the higher liquid losses observed in the emulsion stability analysis (Table 3). In Fig. 3B (EG made with sodium alginate), smaller droplets were observed, with well-defined border around the droplets, which is present in a higher concentration (number of droplets in the same area). However, a considerable increase in the number of droplets with lower diameters were observed in Fig. 3C and 3D corresponding to the EG made with collagen and whey, and alginate and whey, respectively. The size of the oil droplets and their concentration can provide considerable effects on the rheological

properties of the emulsion gels (McClements, 2016), and the images corroborate with the other results discussed in this study.

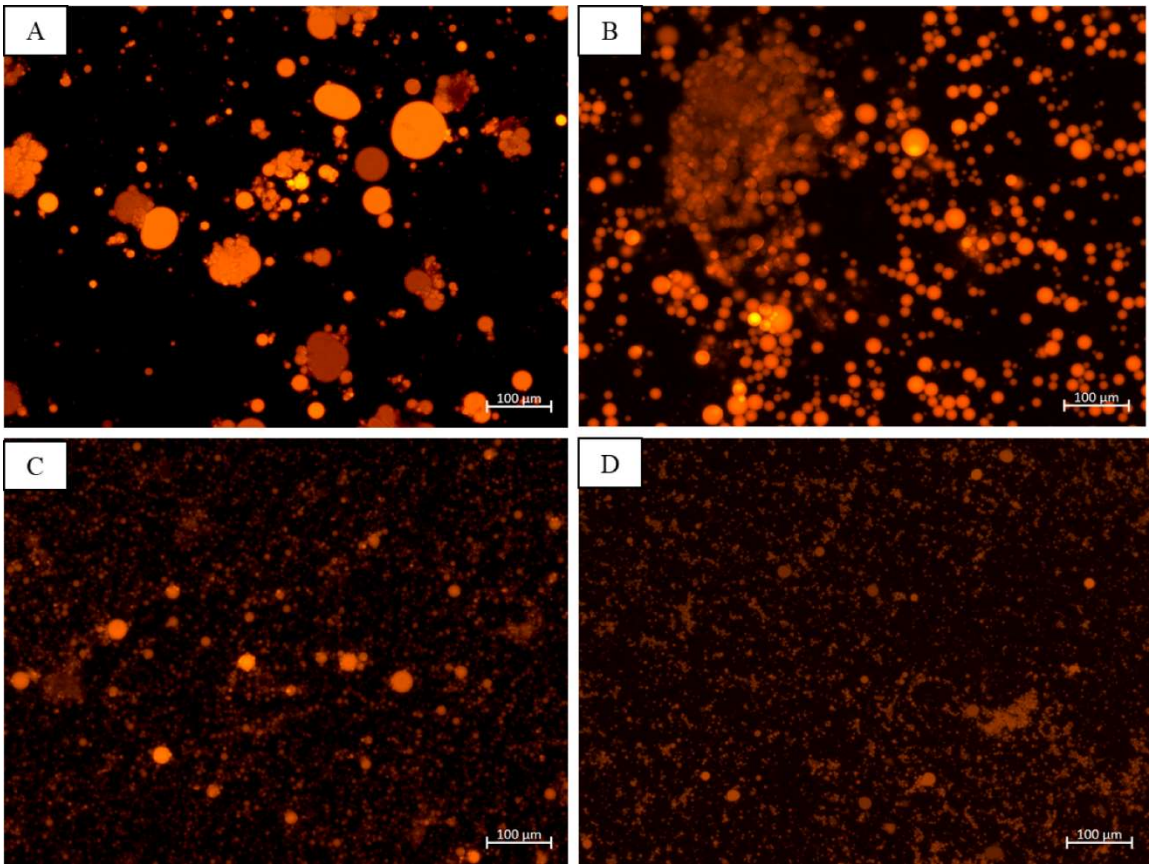

Fig 3. Fluorescence microscopy images of (A) EG made with COL; (B) EG made with ALG; (C) EG made with COL and WHEY; (D) EG made with ALG and WHEY. * 40x magnification. Scale bar: 100 µm.

4. Conclusion

The results showed that the properties of the EGs and meat model systems were affected by the variables selected in this study, with a concomitant effect of the chia mucilage. The PB design showed that MC has a good emulsifying capacity and an inherent capacity for the formation of hydrogels, which when combined with whey protein resulted in greater stability of both EGs and meat model systems. This result was also evidenced by the microstructure images, which demonstrated the presence of a larger number of droplets with smaller diameters, result of the excellent emulsification capacity of this protein. In contrast, the thermodynamic incompatibility in one of the experiments (run 5) demonstrated a lack of chemical affinity between some biopolymers, disclosing unfavorable results for transglutaminase and carrageenan. Probably, blends containing various components can increase the complexity of the system, hindering network interactions. However, MC provided a very favorable technological response when associated with collagen or

sodium alginate. This study contributed to the elucidation of the interactions between chia mucilage, biopolymers, and proteins opening the opportunity to tailoring EGs properties according to desirable application. A compatibility between whey protein, collagen, alginate, and MC was also observed, forming a gelled emulsion system with olive oil, with a notable technological potential for the reformulation of emulsified meat products.

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Table S1. ANOVA referring to the three independent variables (COL, ALG and WHEY) on the responses and their interactions in complete factorial design

	Variation source	Degrees of freedom	Quadratic sum (QS)	Quadratic mean (QM)	Fcalc (95%)	p-value
pH	Model	8	0.12262	0.01533	29.89	0.033
	Linear	3	0.05782	0.01928	37.58	0.026
	Interactions of 2 factors	3	0.00825	0.00275	5.36	0.161
	Interactions of 3 factors	1	0.00006	0.00006	0.11	0.775
	Error	2	0.00103	0.00051		
L*	Model	8	417.598	52.200	53.15	0.019
	Linear	3	322.776	107.592	109.54	0.009
	Interactions of 2 factors	3	8.486	2.829	2.88	0.268
	Interactions of 3 factors	1	0.111	0.111	0.11	0.769
	Error	2	1.964	0.982		
a*	Model	8	8.358	104.475	71.07	0.014
	Linear	3	556.159	185.386	126.11	0.008
	Interactions of 2 factors	3	0.2607	0.0869	5.91	0.148
	Interactions of 3 factors	1	0.0043	0.0043	0.29	0.642
	Error	2	0.0294	0.0147		
b*	Model	8	952.199	119.025	66.97	0.015
	Linear	3	623.826	207.942	117.00	0.008
	Interactions of 2 factors	3	32.016	10.672	6.00	0.146
	Interactions of 3 factors	1	0.0940	0.094	0.53	0.543
	Error	2	0.3555	0.178		
Force penetration (EG)	Model	8	0.7247	0.09059	687.55	0.001
	Linear	3	0.5536	0.18454	1400.53	0.001
	Interactions of 2 factors	3	0.0414	0.01380	104.72	0.009
	Interactions of 3 factors	1	0.0225	0.02250	170.79	0.006
	Error	2	0.0003	0.00013		
G'	Model	8	164926837	20615855	799.64	0.001
	Linear	3	134161778	44720593	1734.60	0.001
	Interactions of 2 factors	3	9216210	3072070	119.16	0.008
	Interactions of 3 factors	1	219089	219089	8.50	0.100
	Error	2	51563	25781		
G''	Model	8	7519684	939960	441.66	0.002
	Linear	3	6147837	2049279	962.89	0.001
	Interactions of 2 factors	3	631108	210369	98.85	0.010
	Interactions of 3 factors	1	1878	1878	0.88	0.447
	Error	2	4257	2128		
Liquid released	Model	8	56.776	7.097	23.24	0.042
	Linear	3	407.327	135.776	44.46	0.022

meat emulsion	Interactions of 2 factors	3	82.968	27.656	9.06	0.101
	Interactions of 3 factors	1	0.016	0.0161	0.05	0.840
	Error	2	0.611	0.3054		

CAPÍTULO 5

OLIVE OIL-BASED EMULSION GELS CONTAINING CHIA (*SALVIA HISPANICA* L.) MUCILAGE DELIVERING HEALTHY CLAIMS TO LOW-SATURATED FAT BOLOGNA SAUSAGES

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Olive oil-based emulsion gels containing chia (*Salvia hispanica* L.) mucilage delivering healthy claims to low-saturated fat Bologna sausages

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Abstract

This study aimed to investigate the effect of adding emulsion gels (EGs) based on chia mucilage (MC) and olive oil while reducing pork back fat (PBF) and phosphate levels by 100% and 50%, respectively, on the physicochemical, sensory, and structural properties of Bologna sausages. The influence of collagen, sodium alginate, and whey protein as gelling agents was also evaluated regarding the technological performance and stability of the emulsion gels, whose combination resulted in 4 treatments. A formulation with 20% of PBF and 0.5% phosphate was prepared as the control (FC). Levels of saturated fatty acids were reduced more than 44% in all treatments with EGs, while the polyunsaturated fatty acids were increased above 56%. High emulsion stability was found in the Bologna sausages with EGs, without significant differences ($P < 0.05$) among all treatments. Regarding textural properties, the samples with EGs were less cohesive and more elastic. The microstructural images showed a change in the structure of the Bologna sausages containing EGs with a less bound protein matrix in the form of a network and less dense, with smaller protein aggregates. However, no significant differences were found in the sensory evaluation ($P < 0.05$) for the texture attributes between treatments. The Bologna sausages reformulated with reduced fat were considered accepted by the consumers, demonstrating that the EGs with MC can be promising ingredients for replacing fats and developing healthier meat products.

Keywords: chia mucilage; emulsion gel; fat substitute; fat reduction; olive oil.

1. Introduction

Recently, there has been growing interest in improving the functional performance of emulsified meat products prepared with reduced fat contents through the use of lipid-phase structural agents, including hydrogels, organogels, and emulsion gels, whose main objective is to act as a substitute for animal fats (Alejandre, Astiasaran, Ansorena & Barbut, 2019; Paglarini et al., 2019; Barbut, Wood & Marangoni, 2016). These systems are capable of incorporating lipophilic functional agents with beneficial health effects on food products, especially in meat products, which traditionally have high levels of saturated fats (Freire et al., 2018).

Emulsion gels (EGs) have been successfully used to replace/reduce animal fat in emulsified meat products (Poyato et al., 2014; Pintado et al., 2018). EGs can be defined as

matrices of polymeric gels (proteins or polysaccharides) in which emulsion droplets are incorporated. These structures are classified as soft solids and can be produced from a stable emulsion incorporated in the continuous gelified phase (Dickinson, 2012; Oliver, Scholten & Aken, 2015). Besides, several studies have reported the use of virgin olive oil to formulate emulsions and meat products in order to design products with improved health benefits due to the presence of high concentrations of monounsaturated fatty acids, squalene, phytosterols, tocopherols, and phenolic compounds (Cofrades, Solas, Herrero, & Jiménez-Colmenero, 2013; Gavahian et al., 2019).

In recent years, the addition of biopolymers and different protein systems to improve the stability of emulsion gels has attracted research attention. Proteins such as whey, collagen, and soy protein and biopolymers such as carrageenan and alginate are generally used to stabilize EGs (Çakir & Foegeding, 2011; Sato et al., 2014; Feng, et al., 2019). Mucilages are functional biopolymers commonly extracted from soft seeds or stems, easily obtained by immersion in water (Kaewmanee, et al., 2014). Chia mucilage (MC) is a high molecular weight polysaccharide extracted from the seeds. When in contact with water, the mucilage exudes from the seed, and the viscosity of the solution increases considerably. This polymer is composed of β-D-xylose, α-D-glucose, and 4-O-methyl-α-D-glucoronic acid in a proportion of 2:1:1, respectively (Lin, Daniel & Whistler, 1994; Muñoz et al., 2012).

Chia mucilage has been described as a new source of polysaccharide gum with various properties that have several potential applications in the food industry (Ali et al., 2012) as a thickening agent or emulsifying agent, and has properties that favor encapsulation by the tendency to form gels in addition to the high water retention capacity (Segura-Campos et al., 2014; Timilsena et al., 2016; Goh et al., 2016). Previous studies also revealed the stabilization properties of emulsions (Capitani et al., 2016; Guiotto et al., 2016).

All the described characteristics reveal a considerable potential of MC to act as a fat substitute, in addition to the possibility of improving texture properties and water retention capacity of meat products in the absence of additives that accomplish this function, like sodium tripolyphosphate. Given these considerations, this study aimed to investigate the effects of adding EGs based on chia mucilage and olive oil to Bologna sausages instead of pork back fat, and reducing the phosphate content by 50% on its physicochemical, sensory, and structural properties. The influence of the gelling agents collagen, sodium alginate, and whey protein was also evaluated regarding the technological performance and stability of the EGs.

2. Material and methods

2.1 Materials

Olive oil designated as an extra virgin (D'Aguirre, Argentina) with 20.68% saturated fatty acids (SFA), 59.61% monounsaturated fatty acids (MUFA) and 19.71 polyunsaturated fatty acids (PUFA), was donated by Sandéleh Alimentos (Sorocaba, Brazil). Chia seeds (*Salvia hispanica* L.) were purchased by Cereal Prime (Cereal Prime, São Paulo, Brazil). Whey protein (90% purity, Alibra Ingredients, Campinas, Brazil), collagen (Novaprom, Guaiçara, Brazil), sodium acid pyrophosphate (ICL Brasil, São Paulo, Brazil), calcium sulfate (Dinâmica Química Contemporânea Ltda, Indaiatuba, Brazil), sodium tripolyphosphate, sodium erythorbate, sodium nitrite and sodium alginate (Kerry do Brasil, Campinas, Brazil) were donated by respective food companies. Nile red dye and fluorescein isothiocyanate (FITC) were purchased from Sigma (Sigma–Aldrich Corporation St. Louis, USA). Pork (*M. longissimus dorsi*) (70.1% moisture, 9.4% lipids, 19.4% protein, and 1.1% ash), beef (*M. gluteus biceps*) (72.5% moisture, 4.6% lipids, 21.5% protein, and 1.4% ash) and pork back fat (10.5% moisture, 80.6% lipids, 8.2% protein, and 0.7% ash) was obtained from a local market in Campinas (Brazil). After cleaning to remove apparent fat and aponeuroses, meat and pork fat were ground in a 5.0 mm disks, packed in vacuum and then frozen (-18 ºC) until further use.

2.2 Extraction of chia (Salvia hispanica L.) mucilage

The mucilage of chia seeds was extracted from whole seeds according to Coorey et al. (2014) and Felisberto et al. (2015) with some modifications. Whole chia seeds were soaked in distilled water (1:25 seed/water volume ratio) for 3 h at 60 °C using an electric cooker (STM, São Paulo, Brazil). The extracted mucilage was separated from the chia seeds using a 35/ CM-876 finisher depulper with a stainless-steel wire mesh of aperture size 0.26 mm (FMC do Brasil Indústria e Comércio Ltda, Araraquara, Brazil). Finally, the mucilaginous gel was dried in an LP 820 freeze-drier (São Paulo, Brazil) and stored in hermetically sealed metalized packaging, protected from moisture.

2.3 Elaboration of the chia mucilage-based emulsion gels (EGs)

The emulsion gel (EG) composition and preparation processes were optimized in previous studies (data not yet published) to obtain various gelled emulsions with the more appropriate physico-chemical properties to replace pork back fat in emulsified meat products. Emulsion gels (EGs) were prepared with 40% olive oil and 5% (as a fixed level) of chia mucilage (MC) together with gelling agents collagen (COL) and alginate (ALG), with or without whey protein (WHEY) (Table 1). For the treatments with whey protein the amount of distilled water (w / w) added in the EGs was 50% and in the other two treatments it was 53%.

Table 1

Description of treatments (%, w/w) of bologna sausages elaborated with chia mucilage-based emulsion gels

Ingredients	Treatments (%)				
	FC	COL	ALG	COL+WHEY	ALG+WHEY
Pork	42	42	42	42	42
Beef	18	18	18	18	18
Pork back fat	20	0	0	0	0
Emulsion gel (EG)	0	20	20	20	20
Description of the EGs	-	*2% of COL, 5% of MC[a], 40% olive oil*	*2% of mixture based on alginate (0.75% ALG, 0.75% $CaSO_4$[b], 0.5% $Na_4P_2O_7$[c]), 5% of MC, 40% olive oil*	*2% of COL, 5% of MC, 40% olive oil, 3% of WHEY*	*2% of mixture based on alginate, 5% of MC, 40% olive oil, 3% of WHEY*
Ice	17.03	17.28	17.28	17.28	17.28
Phosphate[d]	*0.5*	*0.25*	*0.25*	*0.25*	*0.25*

FC – control formulation; COL – collagen powder; ALG – sodium alginate; WHEY – whey protein. [a] MC – chia mucilage; [b] $CaSO_4$ - calcium sulfate; [c] $Na_4P_2O_7$- sodium acid pyrophosphate; [d] Phosphate - sodium tripolyphosphate. The following ingredients and/or additives were also used (%) in each treatment: condiments,0.705; sodium erythorbate, 0.05; sodium nitrite, 0.015; and sodium chloride, 1.7.

The gelling agents collagen were previously dissolved in water at 40 °C and the whey protein was dissolved in water at 70 °C, and cooled to room temperature and then added for homogenization with the remainder of the ingredients. Initially, MC was hydrated with part of the water of the formulation until it formed a paste, which was then homogenized (Thermomix TM5, Vorwerk, Wuppertal, Germany) with water for 90 seconds at 1,100 rpm. After hydration of the mucilage, the gelling agents were added (according to the treatments) and the homogenization was continued for one minute at 4,000 rpm. Olive oil was gradually

incorporated into the mixture for four minutes at 3,500 rpm, and then for one minute at 5,100 rpm. The emulsion gels were placed in polystyrene containers and stored in a refrigerated chamber at 5 °C for 24 hours before preparation of the Bologna sausages.

2.4 Experimental design and preparation of Bologna sausages with EGs

Four low-fat Bologna sausages were prepared with 100% replacement of pork back fat by emulsion gels (EG's) and partial sodium tripolyphosphate reduction (50%), as shown in Table 1. The Bologna sausage prepared with EG with collagen was designated COL, prepared with sodium alginate was designated ALG and the treatments with EGs in combination with whey protein were designated COL+WHEY and ALG+WHEY, respectively. A control formulation (FC) containing 20% por back fat and 0.5% of sodium tripolyphosphate was used as a standard for the traditional Bologna sausage. The levels of EGs, fat, meat and the remaining ingredients raw materials and additives are described in Table 1.

To process the Bologna sausages, ground pork and beef meat (5 mm, 2 ºC), the ingredients salt, half the ice, sodium nitrite, sodium tripolyphosphate, and condiments were placed in the cutter (Mado®, Germany) and comminuted until the temperature reached 7 ºC for extraction of myofibrillar proteins. Then, the remaining ice was added along with the sodium erythorbate and ground pork back fat (5 mm) or emulsion gel (depending on the treatment), followed by comminution until complete homogenization. The temperature of the meat batter did not exceed 12 ºC. The meat batter was stuffed (Mainca®, Spain) into water-impermeable plastic casings of 85-90 mm in diameter (Spel Embalagens, Brazil) with approximately 0.3 kg of product per package. The Bologna sausages were cooked in an oven (Arprotec®, Brazil) according to the following cooking schedule: 15 minutes at 65 ºC and 98% relative humidity (RH), 20 minutes at 75 ºC and 98% RH, and then 85 ºC and 98% RH until the internal temperature reached 72 ºC. The products were then cooled in cold potable water and stored in a cold room (4 ºC) until analysis. About 150 g raw meat batter was sampled from each treatment, placed in plastic tubes and kept at refrigeration temperature (4 ºC) to perform the emulsion stability test. The experiment was carried out in triplicate.

2.5 Chemical composition and energy value

Compositional analysis including moisture, ash and protein content of Bologna sausages was determined according to the methodology described by the Association of Official Analytical Chemists (AOAC, 2005). The nitrogen content was determined by the macro-Kjeldahl acid digestion, and the result was multiplied by 6.25 to determine the protein content. The content of fat was measured following the method of Bligh & Dyer (1959). The determination of phosphates was carried out from the ashes obtained from the samples by spectrophotometric method which is based on the formation of phosphomolybdate with added molybdate followed by its reduction with sodium sulphide in aqueous acidic medium. The readings were made at 420 nm (Instituto Adolfo Lutz, 2005).

Three samples of each replication were carried out and the average values were used for statistical analysis. The energy value was calculated based on 9 kcal/g for fat; 4 kcal/g for protein and carbohydrates.

2.6 Fatty acid profile

Lipid extraction was performed by the Bligh & Dyer (1959) method on the following day after the processing of the Bologna sausages and the esterification was carried out according to Hartman & Lago (1973). The methyl esters of fatty acids were separated according to the Ce-66 method (AOCS, 2009). The samples were analyzed in CGC Agilent 6850 Series GC Capillary Gas Chromatograph, equipped with DB-23 AGILENT (50% cyanopropyl-methylpolysiloxane) capillary column 60 m, Ø int: 0.25 mm, 0.25 μm film. The operating conditions were: column flow = 1.00 mL / min; linear velocity = 24 cm / sec; detector temperature: 280 ºC; injector temperature: 250 ºC; oven temperature: 110 ºC - 5 minutes, 110 – 215 ºC (5 ºC / min), 215 ºC - 34 minutes; carrier gas: helium; volume injected: 1.0 μL; split ratio: 1:50. The fatty acid composition was determined by comparing the peak retention times with those of the respective fatty acid standards. Three samples in each of the three replications of the experiment were analyzed in duplicate and the average of each sample was used in the statistical analysis. The atherogenic index (AI) and thrombogenic index (TI) were calculated according to Ulbricht & Southgate (1991) as a ratio between some SFA and unsaturated fatty acids.

2.7 Technological and physico-chemical properties

The pH of Bologna sausages was evaluated using a MA 130 Mettler pH meter using a penetration probe at different places of the sample. The water activity (aw) was measured by an Aqualab water activity meter (Decagon, Pullman, USA). The percentual of juiciness was measured according to the methodology proposed by Lucherk et al. (2017). Bologna sausage samples were cut in cubic (1 cm) in triplicate and were weighed on 2 sheets of filter paper (Whatman ^{TM}, qualitative, 9.0 cm) previously stored in a desiccator. Samples were compressed for 30 s at 78.45 N with pre-test speed of 3.7 mm/s and post-test of 10 mm/s using a texture analyzer (TA-xT2i Texture Technologies Corp., Scarsdale, NY). After compression, the sample was removed from the filter paper and the filter paper was reweighed. The percentage of fluid weight lost from the sample during compression was quantified as juiciness percentage. Each treatment was evaluated in triplicate, and each analysis was performed at room temperature.

The emulsion stability test was performed according to Hughes et al. (1997), with some modifications. Approximately 25 g raw meat batter of each treatment was placed in plastic tubes and centrifuged for 1 minute at 3600 rpm. Soon after, the samples were heated in a water bath at 70 ºC for 30 minutes and centrifuged again for 3 minutes at 3600 rpm. The total liquid released or the supernatant was placed in 50 mL beakers and oven dried at 100 ºC for 16 hours for complete water evaporation, and weighed to determine the fat released. Five replicates of each treatment were performed, at 24 h after processing.

To evaluate the losses of liquid by exudation (Les) of the sliced samples (commercial presentation, form common in the supermarkets), 8 slices of Bologna sausage of each treatment were weighed, placed in polystyrene trays, without being overlapped and packaged under vacuum and on film of PVC. The trays with the samples were kept at 5 °C for 5 days. After this time, the exudate was withdrawn and the slices were again weighed. The percentage of losses of slices by exudation was calculated for the vacuum samples and for those packaged in PVC film.

The texture properties of the Bologna sausages were analyzed using a Texture Analyzer TA-XT2i (Texture Technologies Corp., Scarsdale, NY) with a compression probe (35-mm diameter) attachment at room temperature. Ten cylinders with 20 mm diameter and 20 mm height were used for each experiment. The samples were compressed to 30% of its original weight, with speed test, pretest and post test of 1.00 mm/s and force of 0.10 N. The parameters

evaluated were hardness (N), springiness, cohesiveness and chewiness (N). The samples were evaluated at 24 h after processing.

The color was measured in a CM-5 spectrophotometer (Konica Minolta, Tokyo, Japan), operating with D65 illuminant, 10° observer angle, SCE mode (regarding sample brightness), and CIELab color system for the evaluation of parameters L*, a*, and b*, values were determined as indicators of lightness, redness, and yellowness, respectively. Three readings of each sample were performed at room temperature (25 °C). Whiteness and ΔE were calculated from the L*, a* and b* values (Park, 1994):

$$\text{Whiteness} = 100 - [\,(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5}$$

$$\Delta E = [L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2]^{0.5}$$, where ΔE is the square root of the sum of squares of the differences between L*, a*, and b* coordinates of the treatments and control formulation.

2.8 Low field NMR analysis

NMR relaxation measurements were performed using a MiniSpec mq 20 NNR spectrometer (Bruker BioSpin, Rheinstetten, Germany) operating at a frequency of 20 MHz. The methods followed those of Bertram, Andersen & Andersen (2007) with some modifications. The samples of Bologna sausages with approximately 1.0 g were directly placed in an 8-mm glass tube and inserted in the NMR probe, and waiting about 15 min was to stabilize the temperature thermalized at 39.8 °C. The T_2 measurements were performed with a τ-value (time between 90° pulse and 180° proton pulses of 8.5 and 16.6 μs, respectively) of 160 μs. The spin–spin relaxation time, t_2, was measured using the Carr-Purcell-Meiboom-Gill sequence. The data, performed were obtained in triplicate in for two different batches samples, from in which 20000 echoes were acquired as with 16 scan repetitions with and the repetition time between subsequent scans of was 15 s. Areas and relaxation times of the relaxation populations found were calculated.

2.9 Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM)

The microstructure of thin slices of the Bologna sausages (three from each treatment) cut out with a scalpel, was studied by confocal laser scanning microscopy (CLSM). Nile red (0.02% w/v in methanol) and Fluorescein Isothiocyanate- FITC (0.02% w/v in acetone)

was used to stain fat and protein, respectively. A Leica TCS SP5II (Leica Microsystems Heidelberg, Germany) equipped with a helium/neon laser was used for the fluorescence excitation (500-530 nm for FITC and 505-586 nm for Nile Red). Data from representative areas for each sample were taken using a 20X magnification objective.

The microstructure of the Bologna sausage too was evaluate using a high vacuum scanning electron microscope TM 3000 Tabletop Microscope, with a magnitude of 15× to 30,000× and a 15 kV acceleration voltage (Hitachi High Technologies, Japan) using a non-destructive technique dispensing preparation of the sample. Magnifications of 200× was used. The product was cut into standard size (2 cm × 2 cm) with a thickness of 2 mm, placed in stub, and analyzed in the modular equipment at 5 kV and 15 kV.

2.10 Sensory analysis

This study was approved by the Ethics in Research Committee of the University of Campinas, SP, Brazil (CAAE – 6815 1217.8.0000.5404) and all participants signed a free and informed consent form, agreeing voluntarily to participate in the sensory tests. A hedonic test was used to evaluate the acceptance of the products for the attributes color, aroma, flavor, texture, overall impression and purchase intent. A three-digit code has been assigned to the samples, which were evaluated by each consumer in a monadic order, following a balanced design as described by Stone, Bleibaum, and Thomas (2012), in individual cabins. A 9-point structured hedonic scale was used, with extremes ranging from "disliked very much" to "liked very much " (Stone & Sidel, 1993). Purchase intention was also evaluated using a 5-point scale (1 = "would certainly not buy" to 5 = "would certainly buy"). For that, 116 participants were randomly recruited among students, staff, and professors at the University of Campinas (Campinas, Brazil). All participants were frequent consumers of Bologna-type sausages.

The participants tasted each sample monadically and filled out the questionnaire. The first part of the questionnaire consumers are asked to first evaluate the attributes of the acceptance test on hedonic scale, and then to complete a check-all-that-apply (CATA) questionnaire with the following 14 attributes: condiments flavor, adequate amount of salt, bologna sausage flavor, little salt, mild flavor, strange residual flavor, salt free, light brown color, pale color, bologna sausage color, bologna sausage aroma, soft texture, succulent, firm texture. Attributes were developed with point of departure on existing sensory literature on processed meat (e.g. Alves et al., 2016; Pires et al., 2019), and later refined and modified based

on qualitative tastings among the authors and collaborators. The consumers were asked to check all of the terms that they considered appropriate to describe each sample. The sensory terms were balanced within and across consumers, following William's Latin Square experimental design (Ares et al., 2010).

2.11 Statistical analysis

Results for all treatments were analyzed by an analysis of variance using a completely randomized design and the general linear model procedure of the Statsoft. Inc. version 7 software (TIBCO Software Inc., California, USA), considering the treatments as a fixed effect, and the experiment replication as a random term (n = 3). Tukey's test at 5% significance level ($P \leq 0.05$) was used to determine significant differences between treatments.

The CATA results were analyzed by frequency analysis of citations for each sensory term of each treatment using XLStat software (version 2018, Addinsoft, Paris, France), Cochran's Q test was performed to identify significant differences between treatments for each term assigned in CATA (Ares & Jaeger, 2013).

3. Results and discussion

3.1 Chemical composition, energy value, and fatty acid profile of Bologna sausages with EGs

The chemical composition and caloric value of the different treatments of Bologna sausages reformulated with EGs are shown in Table 2. The moisture content of the treatments with EGs was higher than FC, control treatment ($P < 0.05$), which is justified by the total substitution of PBF by the EG, which is about 50% water, is an inherent part of the adopted reformulation strategy. Regarding protein content, very similar values were observed since the lean meat content was maintained at a fixed value in all treatments. However, in the treatments with EGs that contained whey protein, protein levels were significantly higher ($P < 0.05$) (COL+WHEY and ALG+WHEY). For phosphate content, the results found in Table 2 show that the reduction was significant ($P < 0.05$) in relation to the FC, however, higher values were obtained for the ALG and ALG+WHEY treatments, which contained sodium pyrophosphate in the EGs, which increased the levels of phosphates.

In all treatments with EGs, fat content decreased at least 43% and caloric value decreased 30% when compared to the FC, allowing the reformulated products to have nutritional claims of "reduced fat" and "reduced energy value" according to the Brazilian legislation and also that of the European Union (Brazil, 2012; EC, 2012). Other published studies show promising results with the use of EGs and organogels to be used as total and/or partial substitutes of fat in meat products, with significant reductions in total fat levels and caloric value (Paglarini et al., 2019; Alejandre et al., 2017; Barbut et al., 2016).

The changes in the nutritional profile of fatty acids were also determined (Table 3). The saturated fatty acid (SFA) levels were reduced by at least 44% in all the reformulated products relative to levels in the control treatment, while the PUFAs increased from 12.5% (FC) to about 20% in the treatments with EGs, an increase over 56%. Also, there was a significant increase in monounsaturated fatty acids (MUFAs) by at least 28% in all reformulated Bologna sausages, justified by the fatty acid profiles characteristic of olive oil, with high levels of oleic acid in its composition, between 55-83% (IOOC, 2013). Pintado et al. (2015) obtained approximately a 40% reduction in SFAs in Frankfurters made with emulsion gels containing olive oil, chia flour, and different gelling agents. Another way to improve the fatty acid profile is the addition of healthier oils such as canola oil through structured systems in the form of organogels. Alejandre et al. (2019) used organogels with canola oil and ethylcellulose to replace animal fat in meat batters and increased the MUFA content of treatments by up to 39%.

Table 2

Technological and physico-chemical properties of Bologna Sausage with chia mucilage-based emulsion gels

	FC[1]	COL	ALG	COL+WHEY	ALG+WHEY
Physico-chemical properties					
pH	6.05±0.01^{b}	6.07±0.01^{a}	6.02±0.01^{c}	6.07±0.01^{a}	5.94±0.01^{d}
Aw	0.976±0.00^{b}	0.979±0.00^{a}	0.979±0.00^{a}	0.980±0.00^{a}	0.978±0.00^{a}
Juiciness (%)	7.16±0.65^{c}	12.30±0.97^{b}	13.10±0.99^{ab}	12.26±0.62^{b}	14.72±0.70^{a}
Emulsion stability					
Liquid exudation (%)	1.69±0.89^{a}	2.17±1.24^{a}	1.86±1.04^{a}	1.84±0.92^{a}	1.31±0.78^{a}
Fat exudation (%)	0.147±0.09^{a}	0.161±0.10^{a}	0.145±0.08^{a}	0.145±0.10^{a}	0.105±0.05^{a}
Liquid exudation of slices (Les) after 5 days (5ºC)					
Les vacuum (%)	2.72±0.19^{bB}	3.67±0.17^{aA}	3.85±0.12^{aA}	3.80±0.30^{aB}	3.91±0.20^{aB}
Les PVC film (%)	3.34±0.02^{cA}	4.22±0.39^{bcA}	5.47±0.15^{abA}	5.79±0.14^{aA}	6.80±0.27^{aA}
Textural parameters					
Hardness (N)	11.14±0.48^{b}	9.87±0.40^{d}	10.43±0.19^{c}	10.36±0.27^{c}	11.68±0.15^{a}
Springiness	0.910±0.01^{c}	0.927±0.00^{ab}	0.922±0.00^{b}	0.930±0.00^{a}	0.927±0.01^{ab}
Cohesiveness	0.811±0.00^{a}	0.812±0.00^{a}	0.800±0.00^{b}	0.808±0.00^{a}	0.803±0.00^{b}
Chewiness (N)	8.32±0.24^{b}	7.54±0.28^{d}	7.68±0.07^{cd}	7.83±0.13^{c}	8.71±0.21^{a}
Chemical composition					
Moisture (%)	64.17±0.11^{c}	72.21±0.02^{a}	72.16±0.02^{a}	71.25±0.01^{b}	71.42±0.07^{b}
Protein (%)	15.21±0.25^{b}	15.24±0.36^{b}	15.01±0.52^{b}	16.11±0.29^{a}	15.89±0.22^{a}
Fat (%)	17.23±0.40^{a}	9.55±0.92^{b}	9.75±0.08^{b}	9.72±0.25^{b}	9.64±0.05^{b}
Ash (%)	2.89±0.05^{a}	2.70±0.02^{b}	2.89±0.04^{a}	2.74±0.01^{b}	2.88±0.01^{a}
Phosphate (%)	0.61±0.02^{a}	0.44±0.01^{c}	0.51±0.02^{bc}	0.46±0.06^{bc}	0.52±0.01^{b}
Energy Value (Kcal/100g)	217.88	148.11	148.52	152.60	150.97
Color parameters					
L*	67.42±0.36^{a}	65.48±0.37^{b}	65.23±0.31^{b}	67.02±0.26^{a}	67.22±0.35^{a}
a*	8.58±0.21^{a}	7.27±0.15^{c}	7.70±0.15^{b}	7.06±0.09^{d}	7.30±0.14^{c}
b*	12.28±0.20^{c}	14.04±0.26^{b}	14.41±0.18^{a}	13.96±0.18^{b}	14.15±0.10^{b}
Whiteness	64.14±0.33^{a}	62.03±0.32^{c}	61.58±0.29^{d}	63.50±0.26^{b}	63.56±0.31^{b}
ΔE	__	2.96±0.19^{a}	3.19±0.28^{a}	2.32±0.13^{b}	2.30±0.13^{b}

* Values represent the average ± standard deviation. All treatments, except FC, has 20% of emulsion gel (EG) with 5% of chia mucilage (MC) and 40% of olive oil. ^{a,b,c,d} Means in the same row with the same letters did not differ significantly at $P < 0.05$. [1] For treatments denominations, see Table 1.

Table 3

Fatty acid profile (%) of Bologna Sausage with chia mucilage-based emulsion gels and/or pork back fat.

Fatty acid	Treatments				
	FC[1]	COL	ALG	COL+WHEY	ALG+WHEY
Myristic C14:0	1.56±0.04[a]	0.33±0.01[b]	0.37±0.01[b]	0.41±0.06[b]	0.38±0.01[b]
Palmitic C16:0	25.72±0.06[a]	18.15±0.13[b]	18.65±0.17[b]	18.65±0.36[b]	18.11±0.14[b]
Stearic C18:0	14.31±0.26[a]	3.67±0.10[b]	4.10±0.05[b]	3.94±0.11[b]	3.67±0.02[b]
Arachidic C20:0	0.28±0.01[b]	0.36±0.01[a]	0.38±0.01[a]	0.37±0.00[a]	0.36±0.01[a]
Others SFA's	1.31±0.04[a]	0.45±0.01[b]	0.52±0.02[b]	0.52±0.06[b]	0.55±0.01[b]
Σ SFA	43.18	22.96	24.02	23.89	23.07
Palmitoleic C16:1	1.96±0.07[b]	2.39±0.08[a]	2.32±0.02[a]	2.38±0.04[a]	2.36±0.04[a]
Margaroleic C17:1	0.32±0.01[a]	0.22±0.02[b]	0.21±0.01[b]	0.22±0.01[b]	0.22±0.02[b]
Oleic C18:1	40.31±0.26[b]	52.61±0.32[a]	52.84±0.48[a]	52.86±0.68[a]	52.84±0.28[a]
Eicosenoic C20:1	0.69±0.01[a]	0.28±0.00[b]	0.29±0.00[b]	0.28±0.00[b]	0.27±0.01[b]
Σ MUFA	43.28	55.50	55.66	55.74	55.69
Linoleic C18:2 (n-6)	12.02±0.11[b]	17.92±0.02[a]	17.10±0.56[a]	17.06±0.08[a]	17.63±0.06[a]
Linolenic C18:3 (n-3)	0.51±0.03[c]	2.95±0.01[a]	2.56±0.04[b]	2.67±0.04[b]	2.93±0.04[a]
Σ PUFA	12.53	20.87	19.66	19.73	20.56
PUFA/SFA	0.29	0.91	0.82	0.83	0.89
n-6/n-3	23.57	6.07	6.68	6.39	6.02
Atherogenic index	0.575	0.255	0.268	0.270	0.258
Thrombogenic index	0.717	0.266	0.287	0.286	0.269
Linoleic C18:2t	0.39±0.01[b]	0.49±0.01[a]	0.49±0.01[a]	0.49±0.00[a]	0.48±0.00[a]
Linolenic C18:3t	0.11±0.04[a]	0.19±0.00[a]	0.18±0.00[a]	0.15±0.04[a]	0.18±0.00[a]
Σ Trans	1.01	0.68	0.67	0.64	0.66

* Values represent the average ± standard deviation. All treatments, except FC, has 20% of emulsion gel (EG) with 5% de chia mucilage (MC) and 40% of olive oil. [a,b,c] Means in the same row with the same letters did not differ significantly at $P < 0.05$. [1] For treatments denominations, see Table 1.

Epidemiological evidence and meta-analyses suggest that, compared to diets with high levels of SFAs, diets rich in MUFAs are associated with a lower concentration of LDL

cholesterol and lower risk of cardiovascular disease (Visioli et al., 2018; Mensink & Katan, 1992; Gardner & Kraemer, 1995).

Additionally, several studies recommend ingesting omega-6/omega-3 fatty acids (n-6/n-3), at a proportion around 4/1, to reduce the risk of many highly prevalent, chronic diseases in Western societies, including cardiovascular diseases, cancer, and inflammatory and autoimmune diseases (Simopoulos, 2004; Tortosa-Caparrós et al., 2017). The results presented in Table 3 show a significant reduction in the ratio of n-6/n-3 from 23.57 in FC to around 6.0 in the treatments reformulated with EGs. Although the reasons (n-6/n-3) found for the samples with EGs have diverged slightly from the recommended values, the proportion of n-6/n-3 is still four times smaller than that of the control formulation. This effect is possibly due to an increase of n-3 in the samples with EGs, since the MC, under the extraction conditions used in this study, has a high lipid value (26%, data not shown), which contains considerable amounts of omega-3 fatty acids (56.3%, data not shown). Atherogenic (AI) and Thrombogenic (TI) indices are also used to measure the propensity of a diet or food to influence the incidence of coronary diseases (Selani et al., 2016). In the same way, as for the other reasons, the use of EGs in Bologna sausages significantly reduced the AI and TI indexes, compared to those of the control group, with reductions of at least 53% and 60%, respectively, in each index. Reductions in AI and TI were observed in the study by Pintado et al. (2015), who found values of 0.55 and 1.36 for control and 0.16 and 0.35 for Frankfurters with EGs with chia flour and olive oil, respectively.

3.2 Technological and physicochemical properties

The physicochemical and technological characteristics of the Bologna sausages with low fat content and EGs are shown in Table 2. The pH values of the samples ranged from 5.94–6.07. In the treatments with sodium alginate, the pH values were significantly lower ($P < 0.05$), possibly due to the addition of sodium acid pyrophosphate in these EGs, however, the results found may be considered acceptable for this type of meat product and agree with other studies that evaluated low-fat Bologna sausages (Paglarini et al., 2019; Alves et al., 2017). The water activity (aw) values found did not differ significantly ($P < 0.05$) among the treatments, but were higher than values for the control, and are related to the highest moisture content observed in the reformulated samples (Table 2).

Substituting PBF with EGs also influenced the water holding capacity (WHC) of the reformulated samples, with this property being directly related to the determination of the percentage of juiciness. We observed significantly higher juiciness percentage values ($P < 0.05$) in the treatments with EGs when compared to the control sample, measured by a methodology adapted for our study with Bologna sausages, that was applied initially to meat. In emulsified meat products, this measure is related to WHC, and the greater the water release, the lower the WHC, and it can be considered a promising objective measure of this property. In the control treatment (FC), there was a lower loss of water, and higher WHC; an understandable result, since in this sample there was a higher percentage of phosphates (0.5%), which act synergistically with sodium chloride assisting in the extraction and solubilization of myofibrillar proteins, responsible for water retention capacity (Desmond, 2006). Among the treatments with EGs, a lower percentage of juiciness was observed in the samples containing collagen, and consequently a higher WHC, as well as a better interaction with the meat matrix. Collagen, mainly in its hydrolyzed form, has been applied to meat products to improve their capacities of gelling and water retention (Prestes et al., 2012; Moraes & Cunha, 2013).

The stability of a meat emulsion is another technological parameter of outstanding importance when measuring the quality of emulsified meat products, and consequently has been used frequently in several studies (Freire et al., 2018; Paglarini et al., 2019; Serdaroğlu et al., 2016). The results shown in Table 2 confirm the high stability of the Bologna sausages produced with EGs, without significant differences between all treatments ($P < 0.05$) regarding the release of liquid and fat exudation. Considering the fact that the EGs contained different components (COL, ALG, and WHEY) that resulted in excellent stability of the meat emulsion, the chia mucilage (MC) played an important role in all EGs. According to Avila-de la Rosa et al. (2015), MC tends to form structured configurations around the oily phase, reflecting its stabilization capacity, which corroborates the results of this study, indicating the enormous potential of this component to provide stability to meat emulsions. The phosphate contents of the formulations of Bologna sausages with EGs were reduced by 50% since the MC present in the emulsion gels has high water retention capacity (Samateh et al., 2018) and could compensate for the reduction of phosphates. High phosphate intake has been greatly criticized for raising the risks of heart disease and be harmful to people with chronic kidney disease, besides being a synthetic additive (Tentori et al., 2008; Ribeiro et al., 1998).

This study also evaluated the water holding capacity (WHC) of the samples sliced in two types of packaging (PVC film and vacuum) commonly used for the commercial

presentation of Bologna sausages. The results obtained (Table 2) demonstrate significant differences ($P < 0.05$) between treatments and between packaging systems. When comparing the packaging systems, less liquid exudation was observed from the vacuum-packed slices, with significantly ($P < 0.05$) lower values in the FC and the treatments containing WHEY. Aspé et al. (2008) mentioned that the pressure difference established in the packaging systems induces the flow of liquid from the meat (in this case the meat product) to the empty volume, and in vacuum packaging, this volume is drastically reduced when compared with that of PVC film. Also, the functional effect of whey protein with elevated WHC (Nicolai et al., 2011) could be highlighted, which contributed to the lower values of exudate liquid from vacuum-packed samples, however, the same effects were not observed in the samples packed in PVC film. Among the treatments, the control formulation had the lowest value of liquid exudation in both packaging systems, which reinforces the fundamental role of sodium tripolyphosphate in water binding capacity, since in the other treatments, there was a 50% reduction of this additive.

3.2.1 Texture profile

The effects of substituting PBF with the EGs and reduction of sodium tripolyphosphate in the texture properties of the samples of Bologna sausages are shown in Table 2. As can be seen, all the textural parameters (hardness, elasticity, cohesiveness, and chewiness) were significantly ($P < 0.05$) affected by the addition of EGs and by reducing the phosphate content by 50%. Similar behaviors for the hardness and chewiness parameters were observed, with higher values for the treatments FC and ALG+WHEY. Animal fat plays a fundamental role in the texture of emulsified meat products and a change in lipid profile, with the decrease of SFAs, as observed in this study (Table 3), brings health benefits but can bring certain losses from a technological point of view, resulting in less firm and stable products. There are very relevant contributions to the texture and final structure of the product when considering the properties of the myofibrillar protein matrix (including the interfacial protein film around the fat globules), as well as the crystallization of the fat (Barbut & Youssef, 2016). In addition, the fundamental role of phosphates, which act synergistically with salt in the extraction of myofibrillar proteins, providing a firmer and more cohesive texture to meat products (Desmond, 2006). Thus, we observed products with lower hardness and chewiness in the treatments COL, ALG, and COL+WHEY. However, for the treatment ALG+WHEY, these parameters were significantly ($P < 0.05$) higher than those in FC, but the values were close.

These results indicate that incorporating the combination of a hydrocolloid with whey protein in Bologna Sausage was able to better mimic the textural properties by compensating for the reductions in PBF and phosphates.

Other studies also report essential advances in textural properties in meat products when animal fat is replaced with emulsion gels, polysaccharides gels, hydrogels, or organogels. Alejandre et al. (2019) demonstrated that substituting bovine fat with hydrogels (canola oil, polysorbate 80, and carrageenan) and organogels (ethylcellulose, canola oil, and glycerol monostearate) in meat batters resulted in products with similar hardness and chewiness to those in the control. Pintado et al. (2015) did not find significant ($P < 0.05$) differences in Frankfurter hardness between control treatments and with EGs containing alginate and gelatin. However, these authors observed higher values of elasticity in the formulations with EGs, corroborating the data found in this study.

Significantly higher elasticity values ($P < 0.05$) were observed in the samples reformulated with EGs when compared with values in the FC. One of the hypotheses refers to Dickinson's (2012) elucidation in his review. A polymer gel formulated with denatured protein (in our case after cooking the products) has some structural characteristics and mechanical properties similar to rubber, in that its network elasticity is dominated by entropic contributions of flexible chains between the cross-links. It can be considered that the elastic properties of the EGs influenced the final texture of the reformulated Bologna sausages.

Regarding cohesiveness, the results showed significantly lower cohesion in samples with EGs based on sodium alginate (ALG and ALG+WHEY) ($P < 0.05$) compared the control and samples containing collagen. Although the samples treated with alginate were firmer than the control, these were also less cohesive; consequently, the interactions were weaker. The characteristics perceived for the EG (data not yet published) are also transmitted to the meat products emulsified with EG in its composition. Thus, it is assumed that the polysaccharides do not entirely cover the oil droplets, contrary to what is observed in protein-stabilized emulsions. In this way, the lack of interaction between polysaccharides and oil droplets inhibits the formation of an adjusted polymeric matrix (Sato et al. 2014; Kim et al., 1999).

3.2.2 Color parameters

A comparison between the products containing EG and control (FC) regarding the colorimetric parameters demonstrated significant differences ($P < 0.05$) for the values of L *, a

* and b * (Table 2), indicating that substituting PBF with EGs does not provide a similar color to the pork back fat in the reformulated Bologna sausages. Regarding luminosity, the treatments COL and ALG had lower L* values and differed from the other treatments ($P < 0.05$). Possibly, this darker tonality of the samples is due to the appearance of the EGs because of the presence of MC (5%), since in the extraction process the seeds are subjected to intense pressure to separate the mucilage, and part of the seed coat may have been incorporated into the MC. The treatments COL+WHEY and ALG+WHEY, although they contained the same amount of MC in their composition, differed by the presence of WHEY protein that promoted an increase in the concentration of smaller fat droplets due to their functional properties of emulsification (Fig 2). The size of the fat globules can be used to explain the differences observed in the luminosity between the treatments since smaller fat globules are related to higher light reflectance (Chanamai & McClements, 2001). Thus, the luminosity did not differ ($P < 0.05$) between the control and samples containing whey protein.

Substituting PBF with EG decreased the values of a* ($P < 0.05$) and increased the values of b* ($P < 0.05$) in all treatments, differentiating from FC. These differences can be explained again by the influence of MC on the EGs, besides being related to the matrices of polysaccharides and olive oil, which can provide a greenish-yellow coloration, as reported by Jiménez-Colmenero et al., 2010. It has been reported that the substituting pork back fat with olive oil reduces a* and increases b* (Ruiz-Capillas et al., 2013; López-López et al., 2009). And, in general, several studies that evaluated the substitution of fat by vegetable oils structured or not with polysaccharides and proteins in meat products also reported the alterations perceived in this study for the parameters a* and b * (Wang et al., 2018; Câmara & Pollonio, 2015; Álvarez et al., 2011). Thus, all treatments presented ΔE values greater than 2, considered a limit by authors Francis and Clydesdale (1975) for visual detection of color alteration.

3.3 Low field NMR analysis

The reformulations of meat products with EGs, in the total absence of pork back fat and adding additional ice, affect the mobility of water and promote transformations in the interaction of their components within the meat matrix, which can affect the quality and yield of the final product. In this study, low field NMR was used to evaluate the changes in mobility of proton signals related to water in the different treatments. According Bertram et al. (2001) the T_{21} component reflects water trapped within the myofibrils, and the T_{22} component

corresponds to water outside the myofibrillar lattice, i.e., extra myofibrillar water in meat or meat product.

The NMR signal decay for all Bologna sausages samples could be fitted into two distinct exponential separate peaks (T_{21} centered at approximately 45-52 ms and T_{22} centered at about 270-310 ms), as shown in Fig.1a, which provide the information how the water is distributed in the sausages. Intriguingly, distributed exponential fitting analysis of T_2 relaxation data on the Bologna sausages revealed a pronounced effect of WHEY protein on the intrinsic water mobility and distribution as the inclusion of WHEY in the emulsion gels resulted in the presence of a distinct water population located in the range between 250 and 275 ms approximately (Fig.1b) (treatments COL+WHEY and ALG+WHEY). In these treatments, there was a considerable decrease in relaxation times, with faster relaxation rates for populations T_{22}. These results demonstrated that the protons of these samples (COL+WHEY and ALG+WHEY) had more restricted mobility. Whey proteins are widely used in the elaboration of emulsion gels, especially due to their high water-binding capacity, thickening, emulsifying and gelling properties (Khalesi et al., 2019; Dickinson, 2012). The reduction of fat and adding EG may have caused reorganization of myofibrillar water into meat product structure. However, the addition of MC probably made this change less abrupt, always maintaining the proportions of both population (T_{21} and T_{22}) around 50% (Fig. 1c).

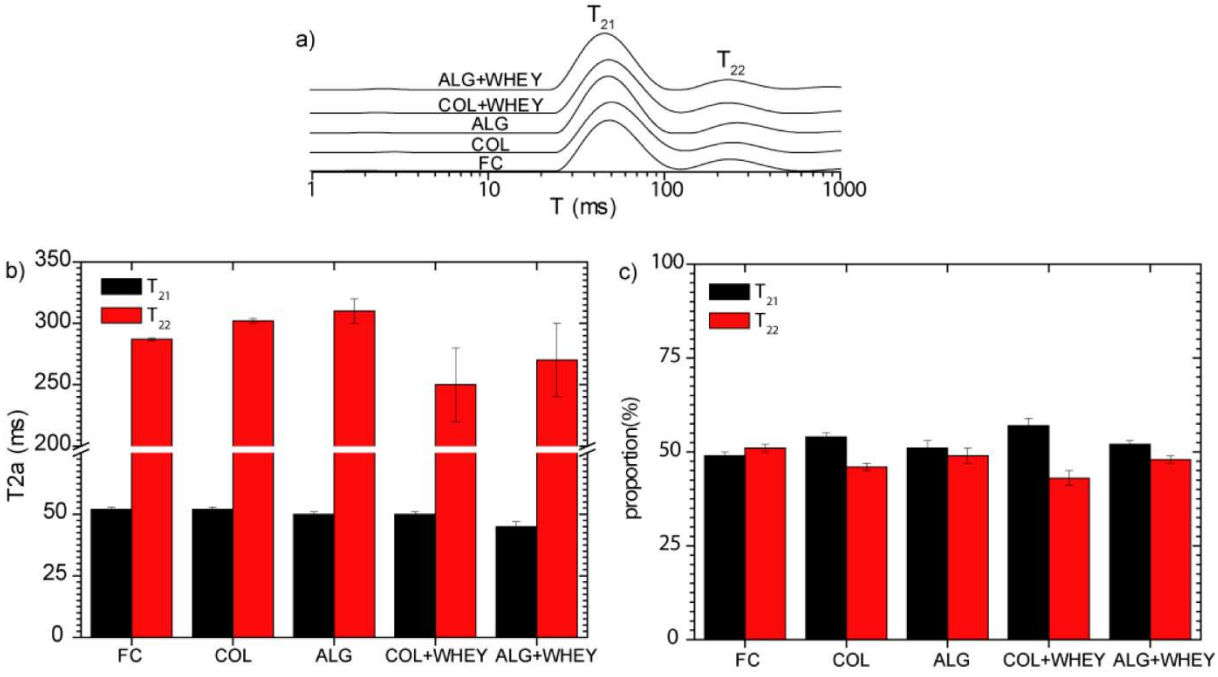

Fig 1. a) Distributions of T_2 relaxation times for Bologna sausages samples with two populations of water referred to as T_{21} and T_{22}. b) T_2 center relaxation times and c) peak areas changes for all treatments, respectively. For treatments denominations, see Table 1.

3.4 Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM)

Images of CLSM and SEM were recorded to evaluate the changes in the structure of the Bologna sausages added of EGs, which totally replaced the PBF in the meat products. Evaluating microstructural images is an effective method to visualize the structure of the protein matrix along with the fat globules in a multicomponent system, as was performed in this study. Fig 2a shows SEM images (left side) and 2b shows CLSM images (right side) of the treatments with EGs, in addition to the control treatment (FC).

In the control treatment, a homogeneous structure was observed with the fat globules uniformly dispersed in a well-adjusted gelified protein matrix, but the fat globules had different sizes, and some were not very well delimited. According to other authors, in meat batter, not all fat particles have a uniform size and shape (Zhang et al., 2013).

However, when EGs are added, changes can be observed in the protein network and in the characteristics of the fat globules. The ALGb and ALGa images and, a lesser proportion COL + WHEYb, show that the protein matrix is no longer a connected and dense network, and is rather more similar to an assembly of smaller protein aggregates, which still bond at some

points but in a less compact way. Larger fat droplets in the COLb and ALGb images can also be observed compared to treatments with whey protein (COL + WHEYb and ALG + WHEYb), however, these are well delimited, sharp droplets, and it is therefore possible to highlight small coalescence points in the ALGb image.

In the images of the COL + WHEY and ALG + WHEY treatments, enough homogeneous distribution of considerably smaller fat droplets in the protein matrix, which reflects greater stability of the system, although previous emulsion stability data (Table 2) demonstrated satisfactory performance of all treatments when compared with FC, with the low release of liquid and fat. Paglarini et al. (2019) observed a more continuous and organized protein matrix in Bologna sausages with EGs prepared with soybean protein, chia flour, inulin, and carrageenan, with the role of chia flour being the most relevant, providing a more compact structure to the samples, which also had lower fluid release and greater stability.

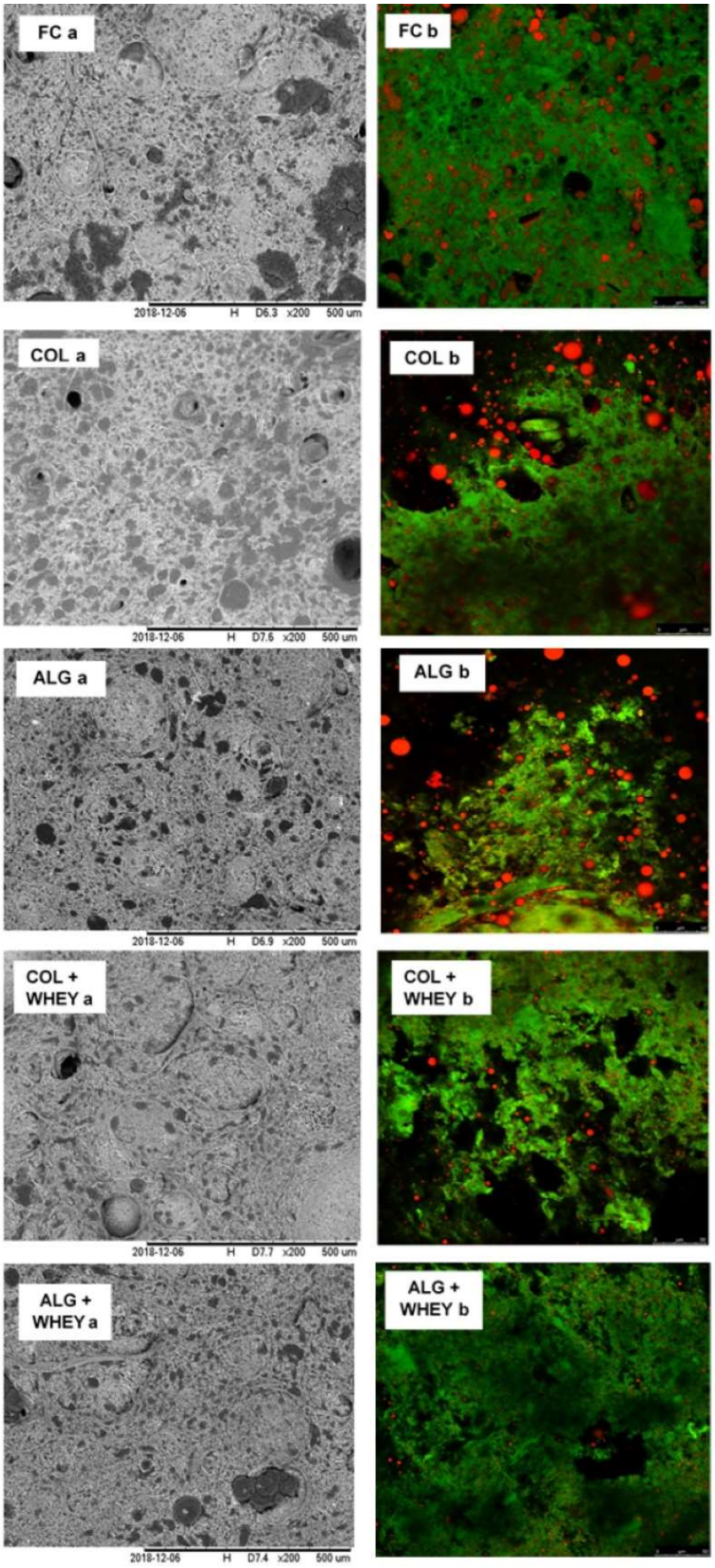

Fig. 2. a. SEM images of Bologna sausage with different EG's or pork back fat (200 x magnification, Scale bar = 500 µm). b. Confocal images of Bologna sausage, same treatments as a. The green, red and black represent protein, oil and air/water, respectively. The scale bar is 50 µm. For treatments denominations, see Table 1.

3.5 Sensory analysis

The results of the hedonic test performed with the consumers, as well as the evaluation of the CATA questionnaire, are presented in Table 4 and Figure 3, respectively. The results were promising and demonstrate the acceptance of consumers concerning the Bologna sausages reformulated with total substitution of PBF by the EGs and a 50% reduction in the phosphate content, without significant differences ($P < 0.05$) in the global acceptance and aroma attributes among all treatments. Regarding the color attribute, the COL+WHEY treatment differed from the others ($P < 0.05$), with this evaluation being directly related to the lowest value found for the instrumental parameter of color a*, which was also significantly lower ($P < 0.05$), reflecting a lower acceptance of this attribute by consumers.

Significant differences ($P < 0.05$) were found for the flavor attribute of the COL treatment, which obtained a lower score for this characteristic when compared with the other treatments. This result may be due to the descriptors that discriminate this sample in the CATA questionnaire (Fig. 3), one being the terms associated with this "salt-free" treatment. As there was a reduction in the levels of phosphate and the levels found for COL were the lowest (Table 2), the consumers may have associated this sample with lower salt content, since in the composition of sodium tripolyphosphate there is about 30% sodium. Pintado et al. (2016) report that the addition of chia flour to frankfurters generated lower scores for the attributes of color, flavor, texture, and global acceptance when compared with the control. Results that highlight the sensory advantages of the use of CM when compared with chia flour, which considerably reduces the unpleasant bitter flavor that flour can provide, a consequence of a large number of phenolic compounds present. According to Ding et al. (2018), the flavonoids account for 80.85% of chia polyphenols, where rutin and hesperidin are the major components.

Another relevant result found in the sensory evaluation refers to the texture attributes. No significant differences were found ($P < 0.05$) for this attribute among all treatments. Results are very desirable, demonstrating that MC incorporated into the emulsion gels, together with the other ingredients (collagen, alginate, and whey protein) were able to provide similar textural characteristics to the control, taking into account the total substitution of PBF by the EGs and a 50% reduction in the sodium tripolyphosphate content. Pinton et al. (2019) observed lower scores for the samples of emulsified meat products with 50% reduction of sodium tripolyphosphate when compared to the control for the attributes of texture, aroma,

and global acceptance, which is in agreement with this study and the success of these reformulations for reducing fat and phosphate contents.

Table 4

Sensory acceptance of Bologna Sausage added of emulsion gels

	FC[1]	COL	ALG	COL+WHEY	ALG+WHEY
Color	6.09±1.71[a]	5.57±1.81[ab]	5.72±1.71[ab]	5.42±1.77[b]	5.53±1.74[ab]
Aroma	6.56±1.65[a]	6.24±1.65[a]	6.35±1.65[a]	6.40±1.67[a]	6.25±1.70[a]
Flavor	6.91±1.54[a]	6.28±1.74[b]	6.34±1.65[ab]	6.44±1.59[ab]	6.55±1.68[ab]
Texture	6.33±1.79[a]	6.33±1.73[a]	6.38±1.59[a]	6.46±1.63[a]	6.59±1.54[a]
Overall acceptance	6.67±1.53[a]	6.15±1.68[a]	6.30±1.53[a]	6.28±1.50[a]	6.45±1.44[a]
Purchase	3.60±1.04[a]	3.15±1.19[b]	3.25±1.05[ab]	3.31±1.05[ab]	3.41±1.01[ab]

* Values represent the average ± standard deviation. All treatments, except FC, has 20% of emulsion gel (EG) with 5% de chia mucilage (MC) and 40% of olive oil. [a,b] Means in the same row with the same letters did not differ significantly at $P < 0.05$. [1] For treatments denominations, see Table 1.

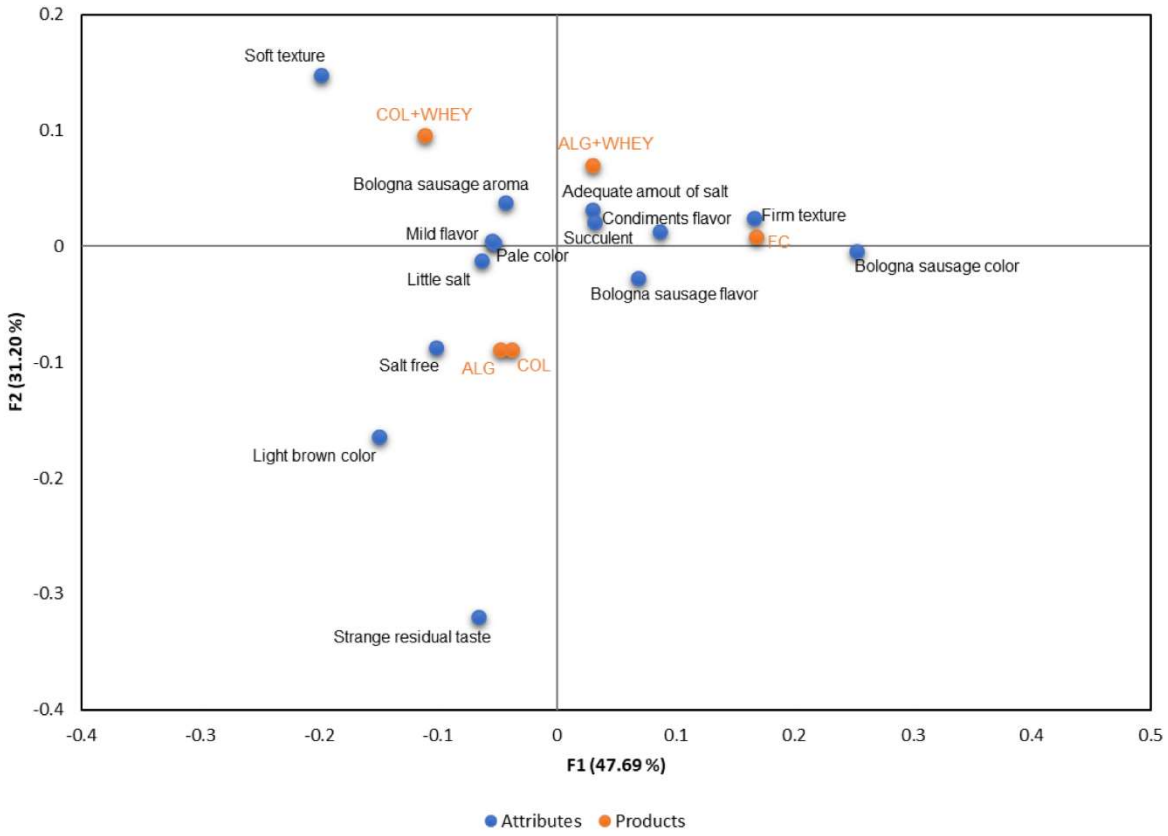

Fig. 3. Representation of the samples and the terms in the first and second dimensions of correspondence analysis performed on check-all-that-apply (CATA) data. All treatments, except FC, has 20% of emulsion gel (EG) with 5% de chia mucilage (MC) and 40% of olive oil. For treatments denominations, see Table 1.

4. Conclusion

Completely replacing pork back fat with emulsions gels of chia mucilage and olive oil, as well as reducing phosphate content by 50%, can be considered technically feasible and nutritionally recommended. This work contributed to a further advance in the reformulations of emulsified meat products, demonstrating that it is possible to produce healthier products with quality characteristics comparable to a control formulation stable meat emulsion, sensory acceptance, and a better profile of fatty acids. The treatments containing whey protein appeared to have been more efficiently incorporated into the meat matrix, exhibiting a more uniform microstructure with more desirable texture and color properties. It is essential to understand the mechanisms of intermolecular interactions between the emulsion gels and the gelled meat matrix so that the most appropriate fat substitutes can be selected and to adjust some characteristics that can still be improved, such as color parameters. Besides that, the study of their stability oxidative would be critical for the successful implementation of this new product.

Declarations of interest

The authors declare that they have no conflict of interest.

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CAPÍTULO 6

SATIETY AND *IN VITRO* DIGESTIBILITY OF LOW SATURATED FAT BOLOGNA SAUSAGES ADDED OF CHIA MUCILAGE AND CHIA MUCILAGE-BASED EMULSION GEL

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Satiety and in vitro digestibility of low saturated fat Bologna sausages added of chia mucilage and chia mucilage-based emulsion gel

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Abstract

The objective of this study was to evaluate the effects of the replacement of pork back fat by chia mucilage (MC) and a chia mucilage-based emulsion gel in Bologna sausages on the appetite sensations of healthy volunteers, and to investigate the microstructural changes of the products during simulated *in vitro* digestion. Three Bologna sausage treatments were made, as follows: FC with 20% pork fat as a control; FMC with 10% fat and 4% rehydrated MC; MC-EG with 100% replacement of pork fat by chia-mucilage-based emulsion gel. The meals were served as breakfast after an overnight fast for 23 healthy young people. Appetite sensations were evaluated by analogous visual scales (VAS) for 120 min. Bologna sausages were submitted to simulated digestion and analyzed by confocal laser scanning microscopy (CLSM) at each phase, and the protein digestibility was determined after the intestinal phase. Similar appetite sensations were perceived by the volunteers ($P > 0.05$) for all treatments throughout the period (1 month). The highest protein digestibility (83.80%) was found for the treatment FC, which differed ($P < 0.05$) from the others. The CSLM images revealed a pronounced aggregation of the samples containing MC in the gastric phase, demonstrating that the formulation may interfere with the bioaccessibility of proteins.

Keywords: in vitro digestion; chia mucilage; satiety; *Salvia hispanica* L; healthier sausage; fat reduction; microstructure.

1. Introduction

Obesity has been a major health problem, once it is directly related to diseases that reduce life expectancy, including cardiovascular diseases. The World Health Organization states that the prevalence of obesity worldwide has more than doubled over the past 40 years (WHO, 2016). In this context, the public interest and media communication has led to a greater demand for reformulated foods with nutritional requirements, associated with a balanced diet (Lundin, Golding & Wooster, 2008). In this scenario, the development of healthier meat products from reformulation strategies, has increased in recent years (Decker & Park, 2010; Han & Bertram, 2017; Cofrades, Benedí, Garcimartin, Sánchez-Muniz & Jimenez-Colmenero, 2017). Although meat products are sources of high biological value proteins, minerals,

vitamins, and mono and polyunsaturated fatty acids, they can also be rich in sodium, additives, and saturated fat, and very poor in fiber, which can justify such reformulations (Feiner, 2006).

However, the fat reduction presents a great challenge, once fat is responsible for the functional properties associated with excellent palatability and sensory characteristics, especially in high-fat foods, as meat products. In addition, the texture, which is greatly affected by the content and type of fat, plays an important role in the development of satiety (McCrickerd & Forde, 2016). Thus, developing healthier meat products with low energy value and high satiating power, by reducing animal fat content and improving the fatty acid profile, is a major challenge since animal fat plays a crucial role in texture, stability, and palatability of these products (Campagnol, dos Santos, Wagner, Terra & Pollonio, 2012). Food fiber from various sources has been used as fat substitutes to provide functional attributes, such as improved texture, emulsion stability, increased gel strength, and better water retention capacity (Talukder, 2015; Zhao, Hou, Cao, Wang, Zhou & Zhang, 2019; Hjelm, Mielby, Gregersen, Eggers & Bertram, 2019).

The addition of dietary fiber in meat product formulations, particularly in emulsified products known to have a high energy value, can result in a reduction in total energy consumption since fiber acts as fat substitutes, besides delaying gastric emptying and increasing the distension of the stomach, which contributes to a greater perception of satiety (Blackwood, Salter, Dettmar, & Chaplin, 2000; Hoad, Rayment, Spiller, Marciani, Alonso, Traynor et al., 2004). Satiety can be defined as the feeling of fullness that persists after eating, suppressing the additional consumption of food (Bellisle, Drewnowski, Anderson, Westerterp-Plantenga & Martins, 2012).

Archer, Johnson, Devereux & Baxter (2004) evaluated whether fat substitution by inulin or lupine seed fiber in burgers affected the perception of satiety in healthy volunteers, and observed higher satiety for the products reformulated with lupine seed fiber. On the other hand, other authors found no differences in the subjective sensations of appetite in meatballs made with pea fiber and rye bran (Kehlet, Pagter, Aaslyng & Raben, 2017).

Chia (*Salvia hispanica* L.) seeds are known to be an excellent source of fiber, besides having expressive amounts of omega-3 fatty acids and proteins (Ayerza & Coates, 2004). Chia seeds in water swell in mucilage (MC), which is a water soluble anionic heteropolysaccharide composed of β-D-xylose, α-D-glucose, and 4-O-methyl-α-D-glucuronic acid in a ratio of 2: 1: 1, respectively (Lin, Daniel & Whistler, 1994). In its isolated form, it presents as a good source of soluble fiber, capable of producing high-viscous dispersions at low

concentrations (Muñoz, Cobos, Diaz, & Aguilera, 2012). In this sense, MC can contribute to the stabilization of emulsified meat products with fat reduction, through the gel formation or continuous phase thickening, resulting in more stable emulsions (Capitani, Corzo-Rios, Chel-Guerrero, Bentacur-Ancona, Nolasco, et al., 2015).

Emulsion gel is defined as an emulsion with a gel-like lattice and solid mechanical properties (Dickinson, 2013). Emulsion gels made with the addition of several functional ingredients as stabilizing agents have been used as fat substitutes in meat products (Pintado, Herrero, Ruiz-Capillas, Triki, Carmona & Jimenez-Colmenero, 2015, Poyato, Ansorena, Berasategi, Navarro-Blasco & Astiasaran, 2014; Paglarini, Martini & Pollonio, 2019). Although the use of MC as a gelling agent in this type of structure is not well elucidated, it may be a successful alternative to mimic the behavior of animal fat in emulsified meat products.

Thus, chia mucilage can provide promising conditions for developing meat products with reduced fat content and functional properties. Reformulating high-fat meat products, such as emulsified products, with real impacts on the regulation of appetite may have an even greater importance since fat is considered the least satiating in relation to the other macronutrients (Blundell & Macdiarmid, 1997; Blundell & Tremblay, 1995).

Although it is possible to find some studies that evaluated the perception of satiety in meat products with fibers, little information is available in the scientific literature about the effects of these reformulations about appetite sensations and the mechanisms involved in digestion, addressing the microstructural and functional properties. In addition, no studies involving chia mucilage, emulsion gels, and the perception of satiety have been reported. Thus, the aim of this study was to evaluate the effects of the substitution of pork back fat by chia mucilage (MC) and the addition of chia-mucilage-based emulsion gel in Bologna sausages on the appetite sensations in healthy volunteers, and to investigate microstructural changes of the products during simulated *in vitro* digestion.

2. Materials and methods

2.1 Materials

Chia seeds (*Salvia hispanica* L.) were purchased by Cereal Prime (Cereal Prime, São Paulo, Brazil). Extra virgin olive oil (D'Aguirre, Argentina) (20.68% saturated fatty acids (SFA), 59.61% monounsaturated fatty acids (MUFA) and 19.71 polyunsaturated fatty acids

(PUFA)), was donated by Sandéleh Alimentos (Sorocaba, Brazil). Calcium sulfate (Dinâmica Química Contemporânea Ltda, Indaiatuba, Brazil), sodium acid pyrophosphate (ICL Brasil, São Paulo, Brazil), sodium tripolyphosphate, sodium erythorbate, sodium nitrite and sodium alginate (Kerry do Brasil, Campinas, Brazil) were donated by respective food companies. Pepsin from porcine gastric mucosa (P6887), pancreatin (P7545) (8 x USP), α-amylase (A3176) from porcine pancreas, nile red dye and fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich (St. Louis, USA). Bile extract from porcine was purchased from ChemCruz™ (Santa Cruz Biotechnology INC, USA). Ultrapure water from a Millipore Milli-Q system (resistivity 18.2 MU/cm) was used for in vitro digestion. The other reagents used in this study were of analytical grade. Pork (*M. longissimus dorsi*), beef (M. gluteus biceps) and pork back fat were obtained from a local market in Campinas (Brazil). After cleaning to remove apparent fat and aponeuroses, meat and pork fat were ground in a 5.0 mm disks and then frozen (-18 ºC) until further use.

2.2 Extraction of chia (Salvia hispanica L.) mucilage

Mucilage extraction was performed according to the method proposed by Coorey, Tjoe & Jayasena (2014) and Felisberto, Wahanik, Gomes-Ruffi, Clerici, Chang & Steel (2015) with some modifications. Briefly, whole chia seeds were soaked in distilled water (1:25 seed/water volume ratio) for 3 h at 60 °C using an electric cooker (STM, São Paulo, Brazil). The mucilage was separated from the seeds using a 35/ CM-876 finisher depulper with a stainless-steel wire mesh of aperture size 0.26 mm (FMC do Brasil Indústria e Comércio Ltda, Araraquara, Brazil). The extracted mucilage was subjected to drying in an LP 820 freeze-drier (São Paulo, Brazil) and stored in hermetically sealed metalized packaging, protected from moisture at room temperature.

2.3 Chia mucilage-based emulsion gel preparation

Emulsion gel was produced according to the procedure describe by Pintado, Ruiz-Capillas, Jiménez-Colmenero, Carmona & Herrero (2015) with some modifications. Emulsion gel (EG) was prepared with 40% olive oil, 53% of distilled water (w / w), and 5% (as a fixed level) of chia mucilage (MC) together with gelling agent alginate (ALG) (0.75% sodium alginate, 0.75 % calcium sulfate, and 0.5% sodium acid pyrophosphate). The composition and

the optimization of the process of elaboration of the emulsion gel (EG) occurred in previous studies (data not yet published) to obtain a product with the most suitable technological properties to replace fat in meat products. First, chia mucilage (MC) was hydrated with part of the water of the formulation until it formed a paste, which was then homogenized (Thermomix TM5, Vorwerk, Wuppertal, Germany) with water for 90 seconds at 1,100 rpm. Soon after, the gelling agent (ALG) was added and the homogenization was continued for one minute at 4,000 rpm. Olive oil was gradually incorporated into the mixture for four minutes at 3,500 rpm, and then for one minute at 5,100 rpm. The emulsion gel was placed in polystyrene containers and kept at 5 º C for 24 hours before Bologna sausage preparation.

2.4 Preparation of Bologna Sausage reformulated with chia mucilage (MC)

Three different Bologna sausages were formulated (Table 1): a control formulation (FC) containing 20% por back fat and 0.5% of sodium tripolyphosphate; a reduced-fat content (10%) with 4% of chia mucilage (rehydrated at 25% concentration in water and kept overnight at 5 ºC) (FMC); a low-fat bologna sausage with 100% replacement of pork back fat by emulsion gel (MC-EG), both with 50% reduction of phosphate. The levels of meat and the remaining ingredients raw materials and additives are described in Table 1.

The Bologna sausages were prepared according to the process described by Paglarini, Furtado, Honório, Mokarzel, Vidal, Ribeiro, Cunha & Pollonio (2019). Partially thawed and ground meat (0 ºC), the ingredients salt, half the ice, sodium nitrite were placed in a cutter (Mado®, Germany) and comminuted until the temperature reached 7 ºC for extraction of myofibrillar proteins. Then, the other additives and ice were added along with the sodium erythorbate and ground pork back fat or emulsion gel or chia mucilage rehydrated (depending on the treatment), followed by comminution until complete homogenization. The temperature of the meat batter did not exceed 12 ºC. After comminution, the prepared sausage mixture was stuffed (Mainca®, Spain) into impermeable plastic casings (90 mm diameter) (Spel Embalagens, Brazil) with 0.3 kg of product per package. The raw sausages were then cooked in an oven (Arprotec®, Brazil) according to the following cooking schedule: 15 minutes at 65 ºC, 20 minutes at 75 ºC and at 85 ºC until the internal temperature reached 72 ºC. The products were then cooled in ice and stored in a cold room (4 ºC) until analysis.

Table 1. Description of treatments (%, w/w) of Bologna sausages elaborated with chia mucilage (MC) and chia mucilage-based emulsion gel (EG)

Ingredients	Treatments (%)		
	FC	FMC	MC-EG
Pork	42	42	42
Beef	18	18	18
Pork back fat	20	10	0
Emulsion gel (EG)[1]	0	0	20
Chia mucilage (MC)	-	4	-
Added ice to rehydrate MC	-	12	-
Ice (total ice)	17.03	23.28	17.28
Sodium tripolyphosphate	0.5	0.25	0.25

FC – control formulation; [1]Emulsion gel – 40% olive oil, 53% water, 5% MC, 2% of mixture based on alginate (0.75% sodium alginate, 0.75% calcium sulfate, and 0.5% sodium acid pyrophosphate). The following ingredients and/or additives were also used (%) in each treatment: condiments,1.155; sodium erythorbate, 0.05; sodium nitrite, 0.015; and sodium chloride, 1.25.

2.5 Proximate composition and energy value

The proximate composition was determined by the official methods of the AOAC (2005), were applied to determine the protein (981.10), moisture (950.46), and ash contents (920.153). The fat was analyzed using the Bligh and Dyer (1959) method. The carbohydrate content was calculated by the difference (100 – [sum of lipid + protein + moisture + ash]). The determination of insoluble and soluble dietary fibers was carried out in the chia mucilage (AOAC, 2012) and estimated in the compositions of the Bologna sausages. Energy content was calculated based on 9 kcal/g for fat; 4 kcal/g of protein and carbohydrate and 2 kcal/g for dietary fiber (EU, 2011).

2.6 Appetite study

2.6.1 Participants

Study participants were recruited from the School of Food Engineering, Campinas. The inclusion criteria were men and women, aging between 18-40 years, BMI 18-30 kg/m^2, and take the habit of consuming meat products at least twice a week. Exclusion criteria were:

food allergy, intolerance to any foods/food ingredients; smokers; regular use of prescription medication; chronic diseases; pregnancy or breastfeeding, participation in diets for weight loss or use of dietary supplements or medications known to interfere in some way with the perception of appetite. All volunteers participated in a short interview to explain the study procedures and completed a questionnaire on issues related to health and consumption habits of meat products. Ethical approval for the study was obtained from the University of Campinas, Brazil ethics committee (CAAE- 68151217.8.0000.5404). Written informed consent was obtained from participants, and they were aware of the possibility of withdrawing from the study at any time they desired. Regarding a sample size, according Blundell et al. (2010), under good experimental conditions, 20-25 subjects is generally sufficient to capture a 10% difference in mean or AUC (area under the curve) appetite ratings between foods. In total, 23 people healthy were recruited and all completed three test meals.

Anthropometric characteristics (weight (kg), height (cm) and waist circumference (cm)), body mass index (BMI), calculated as kg/m2 (with height and weight measurements) were taken (Table 2). Body composition was measured using the bio-impedance method (BC-418MA, TANITA).

Table 2. Baseline subject characteristics (n = 23)

Characteristics	Mean ± SD
Age	27 ± 2.5
Body mass index (kg/m$^{2)}$	23.1 ± 3.7
Body fat (%)	28.5 ± 5.9
Males	21.7 ± 7.8
Females	29.6 ± 5.2
Waist circumference (cm)	82.5 ± 9.7
Males	82.1 ± 11.1
Females	81 ± 9.6

2.6.2 Test meals

The three meals were served in sensory booths as breakfast meals, and their nutritional composition is presented in Table 3. Each meal consisted of 75 g of Bologna sausage, 50 g of bread and 200 mL of water. Energy and macronutrient intake of consumed

breads were calculated by using the Food Composition Database for Epidemiological Studies in Brazil (TACO, 2011). The Bologna sausages were stored at 5 ºC until further use.

Table 3. Proximate composition and energy value of different Bologna sausage formulations and nutritional composition of test meals proposed to volunteers.

	FC[1]	FMC	MC-EG
Proximate composition of Bologna Sausages (%)			
Moisture	62.79±0.09[c]	66.81±0.03[b]	70.65±0.03[a]
Protein	15.94±0.59[ab]	16.57±0.12[a]	15.63±0.13[b]
Fat	17.10±0.66[a]	11.47±0.16[b]	9.49±0.13[c]
Ash	2.79±0.04[a]	2.55±0.02[b]	2.84±0.01[a]
Carbohydrates[2]	1.39	2.60	1.40
Dietary fiber[3]	-	1.53	0.40
Energy value (Kcal/100g)	223.17	183.01	154.30
Nutritional composition of test meals (%)			
Protein	13.76	14.16	13.6
Fat	11.6	8.2	7.02
Carbohydrates	30.67	31.4	30.42
Dietary fiber	1.36	2.28	1.6
Bologna sausage (g)	75	75	75
Bread (g)	50	50	50
Energy value (Kcal)	355.9	325.76	304.23

[a,b,c] Means in the same row with the same letters did not differ significantly at $P < 0.05$. [1] For treatments denominations, see Table 1. [2]Carbohydrates (%) =100%-moisture (%)-crude fat (%)-crude protein (%)-ash (%). [3]Values by calculation. Dietary fiber content: Chia mucilage 38%.

2.6.3 Study design

The appetite study had a single-blind randomized cross-over design was used to investigate the satiating effects of three treatments (FC, FMC and MC-EG) with substitution of pork back fat by chia mucilage (rehydrated or emulsion gel). Viscous and soluble dietary fibers such as oilseed mucilages can delay gastric emptying and decrease the rate of fat and glucose absorption leading to a longer satiety sensation (Kristensen, Savorani, Christensen, Engelsen, Bügel, Toubro, Tetens & Astrup, 2013). Study was carried out at School of Food Engineering from January to February 2019. Study participants were randomly assigned to a sequence of the three test meals using a randomized block design. Participants were instructed to abstain

from alcohol and physical activity for 24h prior to the study day. On each study day, subject arrived in a 10 h fasting state in the morning. Appetite sensations were assed using visual analogue scales (VAS) in the fasting state. Afterward, the meal was served, and participants were told to consume within 15 minutes. After consumption of the test meal, appetite sensations were assessed continuously for 2 h in total (15 min, 30 min, 45 min, 60 min, 90 min, and 120 min). Subjective appetite profile was measured with the use of scales 100 mm VAS (Flint, Raben, Blundell & Astrup, 2000). Appetite profiles measured include ratings of 'hunger' (How hungry do you feel?), 'desire to eat' (How strong is your desire to eat?), 'satiety' (How satiated [i.e. pleasantly satisfied] are you?), 'fullness' (How full do you feel?), and 'prospective consumption' (How much food do you think you could eat right now?), all anchored by the terms 'not at all' and 'extremely' (Blundell, De Graaf, Hulshof, Jebb, Livingstone, Lluch, et al., 2010; Flint et al., 2000).

To examine the sensation ratings or appetite profile over time, the incremental areas under the curves (iAUC) calculated using the trapezoidal rule were used. The analysis was performed with normalized data (compared to basal values). The satiety efficiency of the Bologna sausage was assessed by the satiety quotient (SQ). SQ, expressed as cm/kcal, is an index obtained by the ratio between appetite sensation (AS) scores and energy intake, developed to assess the satiating effect of a test meal standardized for unit of intake (weight or energy) (Green, Delargy, Joanes & Blundell, 1997), using the following formula:

Satiety quotient = [(post-meal AS – pre-meal AS (fasting state))/ energy content of test meal (kcal)] x 100

The same formula is used to calculate SQ for satiety and fullness (post-as – pre-as/kcal). However, in order to calculate SQ for hunger, prospective consumption and desire to eat, the formula needs to be swapped (pre-as – post-as/kcal) to provide positive values of SQ. In all cases, SQ values will be interpreted the same way.

2.7 In vitro digestion and protein digestibility of Bologna sausages reformulated

Samples of Bologna sausages were ground in food processor (GM 200, Retsch, Germany) at 5000 rpm for 20 seconds and were exposed to digestion conditions by subjecting them to sequential incubation in simulated salivary fluid (SSF), simulated gastric fluid (SGF)

and then in simulated intestinal fluid (SIF) using the slightly modified in vitro digestion protocol by Minekus, Alminger, Alvito, Ballance, Bohn & Bourlieu et al. (2014). Sausage samples (3 g) were placed in a beaker with controlled temperature (37 ± 1 °C) and stirring rotation (100 rpm) using a magnetic stirrer (MR Hei-Tec Ø 145- Heidolph Instruments GmbH & Co, Germany). Then, samples were incubated for 2 min with SSF and amylase (75 U/mL) at pH 7. Subsequently, samples + SSF were mixed with SGF at pH 3 (SGF containing 6.9 mmol L^{-1} of KCl, 0.9 mmol L^{-1} KH_2PO_4, 25.0 mmol L^{-1} $NaHCO_3$, 47.2 mmol L^{-1} NaCl, 0.1 mmol L^{-1} $MgCl_2(H_2O)_6$, 0.5 mmol L^{-1} $(NH_4)_2CO_3$, 0.15 mmol L^{-1} $CaCl_2(H_2O)_2$ and freshly prepared pepsin dispersion (25,000 U mL^{-1}) and maintained in the system for the time corresponding to the gastric stage (2 hours). After gastric digestion was completed, the sample + SSF + SGF was further mixed (1:1) with SIF. The temperature was adjusted to 37 ± 1 °C and pH adjusted to 7 with 1M NaOH. SIF was comprised of 6.8 mmol L^{-1} KCl, 0.8 mmol L^{-1} KH_2PO_4, 85.0 mmol L^{-1} $NaHCO_3$, 38.42 mmol L^{-1} NaCl, 0.33 mmol L^{-1} $MgCl_2(H_2O)_6$, 0.6 mmol L^{-1} $CaCl_2(H_2O)_2$ and 70.72 g L^{-1} of bile salts. The fresh pancreatin dispersion (2,000 U mL^{-1} based on tripsin activity) was also added to the system. The mixture was maintained at 37 °C with continuous stirring for 120 min. Sampling was carried out at the end of each stage of the digestion (oral, gastric and intestinal). To stop the enzymatic reactions after the different sampling times along the oral, gastric and intestinal simulation digestion, the tubes were placed in ice for 10 min and frozen at -40 ºC until analysis.

An aliquot of the sample digested, at the end of the digestion steps, was placed in an ice bath and then centrifuged at 10000 g at 4 °C for 20 min to determine the protein digestibility, according to a procedure described by Lin, Chen, Tu, Yu, Dai & Shen (2019) with minor modifications.The supernatant was used for measuring the % nitrogen content (B) by Kjeldahl analysis. The protein digestibility was calculated as following:

PD (%) = B/A x 100, where A is the total nitrogen content (%) of the sample before digestion

2.8 Confocal laser scanning microscopy (CLSM)

A Leica TCS SP5II (Leica Microsystems Heidelberg, Germany) equipped with a helium/neon laser was used for the fluorescence excitation (500-530 nm for FITC and 505-586 nm for Nile Red). Nile red (0.02% w/v in methanol) and Fluorescein Isothiocyanate- FITC (0.02% w/v in acetone) was used to stain fat and protein, respectively. A small aliquot of the sample was placed in the central microscope slide, then, ten microliters of each dye was added,

and the cover slide was carefully positioned to exclude air pockets. The observations were made 10 min after diffusion of the dyes into the sample. For the Bologna sausages samples prior to digestion, thin slices were cut with a scalpel and stained with the same dyes (ten microliters) of the digested samples. Data from representative areas for each sample were taken using a 20X magnification objective.

2.9 Statistical analysis

Analysis of variance (ANOVA) was performed on the data using of the Statsoft. Inc. version 7 software (TIBCO Software Inc., California, USA). Tukey's test at 5% significance level ($P < 0.05$) was used to determine significant differences between treatments.

In the appetite study, the effect of meal on satiety, hunger, fullness, and prospective intake was investigated using repeated measurements as well as a summary measure: incremental area under the curve (iAUC) calculated using the trapezoidal method. ANOVA and Tukey's test for multiple comparison of data related to iAUC and SQs were performed using GraphPad Prism software version 5 (GraphPad Prism 5, California, USA). Level of statistical significance was set to $P < 0.05$.

3. Results and discussion

3.1 Composition and energy value of Bologna sausages and test meals

The proximate composition and the energy value of the Bologna sausages and test meals are shown in Table 3. The reformulation of the Bologna sausages with chia mucilage or chia mucilage-based emulsion gel led to a reduction of fat content and, consequently, the energy value of the products. Significant differences ($P <0.05$) were also found in the moisture contents of the samples. A reduction of 32.92% of fat content and 18% of energy value was observed for the chia mucilage containing (FMC), whereas the treatments containing MC-based emulsion gels (MC-EG) were 44.50% lower in fat content and 30.9% lower in energy value when compared to the control (FC). Therefore, the treatments FMC and MC-EG may have nutritional claims of "reduced fat" according to the Brazilian Resolution RDC 54/2012 (Brazil, 2012) and European Regulation 1924/2006 (EC, 2006), once they meet the requirements of 25 and 30% of fat reduction, respectively, when compared to conventional products (FC). The differences

in fat and energy levels of the Bologna sausages reflected the same differences for the breakfast test meals.

In relation to the moisture contents, the highest values of the treatments FMC and MC-EG treatments can be attributed to the balance of the ice formulation due to fat reduction (FMC) and the water content present (53%) in the emulsion formulation gel used in MC-EG. Similar results were reported by Paglarini, Martini & Pollonio (2019), who used emulsion gels to replace fat in Frankfurters, and reported higher moisture values for the reformulated sausages when compared to the control.

3.2 Appetite sensations after the consumption of the Bologna sausages

Fig. 1 shows the curves of the appetite sensations for hunger, satiety, prospective consumption, desire to eat, and fullness over time and the related iAUC values. Similar results were observed for the appetite sensations evaluated by the volunteers ($P > 0.05$) for all treatments during the whole period (120 min), demonstrating no differences for the meals containing the Bologna sausages reformulated with chia mucilage and chia-mucilage based emulsion gel when compared to the control.The satiety quotient (SQ) analysis was performed to contribute to the understanding of the effects of product formulations on the appetite sensations studied. The quotients in Table 4 agree with the iAUC data, except for the feeling of satiety at times 30 and 45 min, which presented higher ($P < 0.05$) satiety-related quotients for the FMC and MC-EG treatments in comparison with the control (FC).

The initial hypothesis was that the addition of chia mucilage (MC) to the Bologna sausages, directly or in the form of emulsion gel, may have induced satiety when compared to the control treatment. The chia mucilage was composed of soluble and insoluble fibers due to the extraction technique used in this study, and had the ability to form gels, thus leading to a delay in gastric emptying, which is associated with the increase in the perception of satiety (Timilsena, Adhikari, Kasapis, & Adhikari, 2015; Taghipoor, Barles, Georgelin, Licois, & Lescoat, 2014). In addition, the inclusion of sodium alginate in the MC-EG treatment, an algae-derived fiber reported as an appetite modifying compound in acute feeding models (Paxman, Richardson, Dettmar & Corfe, 2008) also supported the initial hypothesis.

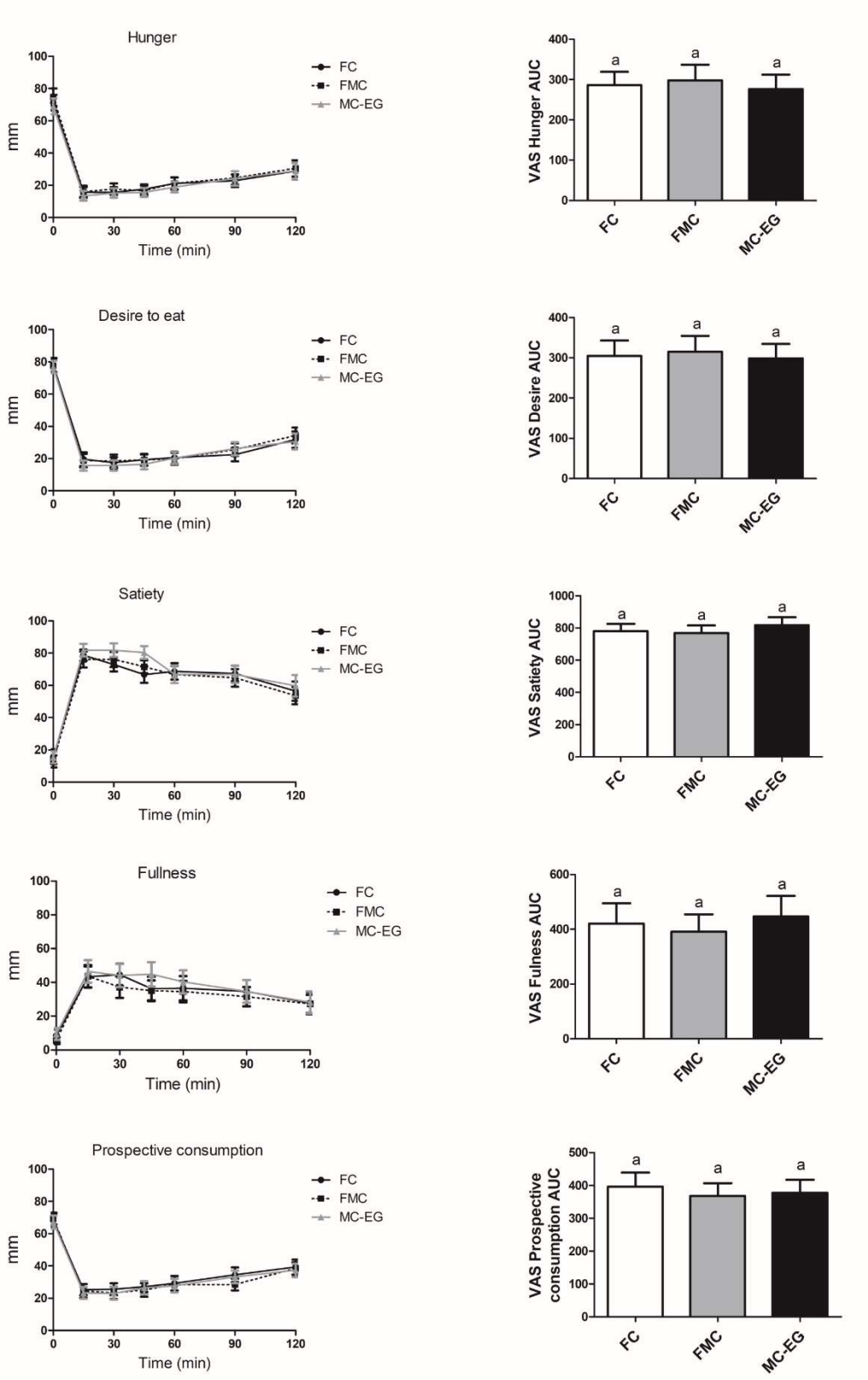

Fig. 1. Variations of the 'hunger', 'prospective consumption',' desire to eat', 'satiety', 'fullness', and 'prospective consumption'parameters at fasting (T0) and after consumption of breakfast (T15, T30, T45, T60, T90 and T120 min) in healthy volunteers (n = 23). For treatments denominations, see Table 1.

Table 4. Satiety quotients related to hunger, satiety, prospective consumption, desire to eat and fullness sensations for Bologna sausages. Values are expressed as mean ± SD.

Sensation (cm/kcal)		FC[1]	FMC	MC-EG
Hunger	15[2]	1.56±0.81[a]	1.80±0.85[a]	1.82±0.89[a]
	30	1.56±0.78[a]	1.75±0.87[a]	1.77±0.86[a]
	45	1.51±0.76[a]	1.78±0.88[a]	1.76±0.74[a]
	60	1.41±0.84[a]	1.64±0.92[a]	1.65±0.73[a]
	90	1.36±0.89[a]	1.54±0.94[a]	1.47±0.85[a]
	120	1.19±0.99[a]	1.36±1.02[a]	1.32±0.87[a]
Satiety	15	1.77±0.71[a]	1.94±0.91[a]	2.18±0.70[a]
	30	1.61±0.77[b]	1.94±0.82[ab]	2.18±0.80[a]
	45	1.43±0.88[b]	1.80±0.90[ab]	2.14±0.71[a]
	60	1.49±0.86[a]	1.65±1.05[a]	1.91±0.82[a]
	90	1.45±0.84[a]	1.59±1.00[a]	1.69±0.89[a]
	120	1.14±0.93[a]	1.25±0.94[a]	1.46±1.00[a]
Prospective consumption	15	1.21±0.54[a]	1.38±0.72[a]	1.44±0.79[a]
	30	1.20±0.58[a]	1.42±0.69[a]	1.45±0.90[a]
	45	1.16±0.63[a]	1.36±0.74[a]	1.34±0.88[a]
	60	1.10±0.69[a]	1.37±0.70[a]	1.30±0.89[a]
	90	0.95±0.63[a]	1.26±0.72[a]	1.12±0.77[a]
	120	0.82±0.74[a]	0.95±0.60[a]	0.98±0.74[a]
Desire to eat	15	1.64±0.76[a]	1.81±0.81[a]	2.01±0.66[a]
	30	1.70±0.73[a]	1.81±0.84[a]	2.01±0.71[a]
	45	1.65±0.74[a]	1.79±0.81[a]	1.98±0.70[a]
	60	1.62±0.76[a]	1.77±0.84[a]	1.86±0.68[a]
	90	1.56±0.86[a]	1.60±0.90[a]	1.67±0.77[a]
	120	1.30±0.92[a]	1.33±0.92[a]	1.52±0.81[a]
Fullness	15	1.12±0.74[a]	1.34±0.81[a]	1.43±0.66[a]
	30	1.15±0.73[a]	1.11±0.71[a]	1.35±0.70[a]
	45	0.90±0.54[a]	1.03±0.81[a]	1.37±0.70[a]
	60	0.89±0.56[a]	1.00±0.62[a]	1.21±0.68[a]
	90	0.85±0.52[a]	0.90±0.70[a]	1.00±0.88[a]
	120	0.62±0.85[a]	0.71±0.66[a]	0.75±0.81[a]

[a,b] Means in the same row with the same letters did not differ significantly at $P < 0.05$. [1] For treatments denominations, see Table 1. [2]Appetite sensations were assessed for 2 h in total, using visual analogue scales (VAS) (15 min, 30 min, 45 min, 60 min, 90 min, and 120 min).

However, it is difficult to predict the interaction between these fibers and a complex protein matrix, such as emulsified meat products, and the impact of these formulations on both the appetite and sensory perceptions. Thus, the absence of differences in feelings of satiety could have more than one hypothesis to explain it.

The present results may be due to the differences in total fiber content of the breakfast meals, which is small when compared to other studies (Brum, Gibb, Peters & Mattes, 2016; Vuholm, Jakobsen, Sørensen, Kehlet, Raben & Kristensen, 2014). Brum et al. (2016) reported that a 6.8 g dose of psyllium provided more consistent satiety benefits when compared to placebo, with a significant decrease in feelings of hunger and desire to eat, as well as an increase in fullness. Vuholm et al. (2014) also observed a decrease in appetite sensations in sausages containing 10 g of dietary fiber. However, in the present study, the meal with the highest amount of fiber (FMC) was only 2.3% and was therefore considered a lower level than in other studies.

Minor differences in fiber contents may induce the effects of satiety early, as observed in the highest satiety quotients at times 30 and 45 min for the treatments FMC and MC-EG (Table 4), but may be less relevant in the perceptions of delayed satiety. Previous studies suggested that fiber intake needs to be increased by about 14 g per day for at least two days to reduce the energy consumption by 10% (Howarth, Saltzman & Roberts, 2001). However, the use of higher fiber concentrations in meat products can lead to technological limitations, such as perceptible changes in texture, color, palatability, and stability (Hjelm, Mielby, Gregersen, Eggers & Bertram, 2019; Schmiele, Mascarenhas, Barretto & Pollonio, 2015).

On the other hand, the results evidenced similar satiety perceptions for the reduced-fat formulations containing chia mucilage (MC) and chia mucilage-based emulsion gel (MC-EG). Whereas a significant reduction of the energy value and saturated fat was observed in both treatments from a nutritional point of view, these results are particularly favorable and may contribute to a healthier consumption of this category of products.

Another relevant issue is related to the limitations of the methodologies that investigate satiety, including physiological and cognitive factors in the perception of this attribute, which is susceptible to a series of extrinsic and individual parameters (Livingstone, Robson, Welch, Burns, Burrows & McCormack, 2000). The satiety ratings of the VAS scales do not always correlate significantly with food intake and energy intake (Stubbs, Hughes, Johnstone, Rowley, Reid, Elia, et al., 2000). VAS scales are subjective tools that are sensitive

to individual interpretations. The sensory and cognitive signals generated by the visual and sensory characteristics of a food influence the consumers' behavior, as well as several factors that affect these responses, including palatability, and cultural and social factors, stress level, and sleep habits (Hetherington, 2007; Morseli, Leproult, Balbo & Spiegal, 2010; Lemmens, Rutter, Born & Westerterp-Plantenga, 2011). In addition to these variables, the sensory differences of the Bologna sausages made with MC (FMC and MC-EG) may have influenced the responses of the volunteers. Thus, further studies are required with this category of processed product, once emulsified meat products have been associated with unhealthy food, despite being rich in proteins of high biological value, minerals, particularly iron and zinc, and B vitamins. Besides, the emulsified meat products are characterized by its practicality, convenience, stability, and low cost, being able to reach a class of consumers that can benefit from its consumption, nevertheless with attributes of composition more favorable to adequate nutrition.

3.3 Protein digestibility in vitro

Protein digestibility together with bioavailability and amino acid composition are the most important factors to establish the protein quality of foods, intrinsically associated with the nutritional quality. The digestion process includes mechanical, chemical and enzymatic steps to release nutrients and facilitate their absorption (Rinaldi, Rioux, Britten, & Turgeon, 2015). The effects of the substitution of pork back fat by chia mucilage using different forms of addition (FMC and MC-EG) on the protein digestibility of the Bologna sausages through the simulation of the gastrointestinal digestion are shown in Fig. 2. The highest digestibility value (83.80%) was found in the control formulation (FC), which differed significantly ($P<0.05$) from the treatments FMC and MC-EG, with 68.73% and 64.24%, respectively.

The results demonstrate that both the chia mucilage and the MC-based emulsion gel interfered in the protein digestibility of the products. In addition, the digestibility values decreased with the decrease in the animal fat content ($P<0.05$). The susceptibility of meat proteins to the action of enzymes during the gastrointestinal digestion depends on structural changes that can affect the active sites of proteases (Simonetti, Gambacorta & Perna, 2016). Concerning the chia mucilage, *in vitro* digestion simulation studies have shown that the apparent viscosity in the stomach increases as a function of chia mucilage concentration, occurring a delay in the gastric emptying (Lazaro, Puente, Zúñiga & Muñoz, 2018; Tamargo,

Cueva, Laguna, Moreno-Arribas & Muñoz, 2018). It is assumed that this behavior reduces the movement of molecules and produces an even more dense and compact structure that hinders the access to enzymes and digestive juices (Lazaro et al., 2018).

It is important to consider the effects of the emulsion gel, which has sodium alginate in its composition, on the protein digestibility, besides the chia mucilage that can also affect the digestive processes, delaying gastric emptying (Spyropoulos, Norton, & Norton, 2011).

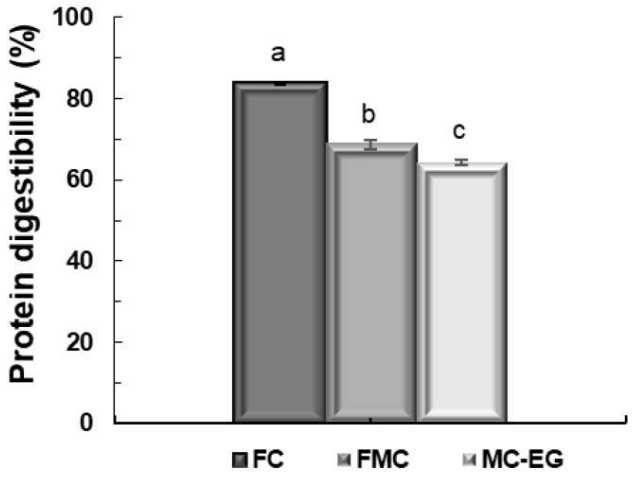

Fig. 2. Effects of chia mucilage (MC) and emulsion gel with MC on in vitro digestibility of Bologna sausages. For treatments denominations, see Table 1.

Soukolis, Fisk, Bohn & Hoffmann (2016) evaluated the ability of sodium alginate to be structured in the intragastric environment under simulated conditions of the gastrointestinal tract, and reported that some typical characteristics of the organism such as ions and pH might interfere in this structure, but still it occurs, and may promote a gastric distension and signal higher perception of satiety. The interaction between sodium alginate and other biopolymers such as MC may have formed a high-viscous structure that certainly interfered with the performance of the proteases, consequently decreasing the protein digestibility of Bologna sausages made with emulsion gel (MC-EG).

Although the reformulation of Bologna sausages has led to a decrease in protein digestibility, it is worth mentioning the health benefits of fiber-rich diets, including increased retention time in the stomach, which may result in early satiety, besides the capacity to attenuate the postprandial glycemia (Soukoulis, C., Gaiani, C., & Hoffmann, 2018). The confocal microscopy images can complement the protein digestibility results and assist in the elucidation of the mechanisms involved.

3.4 Confocal Laser Scanning Microscopy (CLSM)

During digestion, the structural changes in the Bologna sausages due to the differences in ionic strength, pH, enzyme performance, and digestive juices were recorded by image acquisition during the different digestion steps (oral, gastric, and intestinal). Figure 3 shows the images captured before (3A1, 3B1, 3C1) and after each digestion phase (3A2-3A4; 3B2-3B4; 3C2-3C4), with A corresponding to the images of the control formulation FC, B referring to FMC, and C corresponding to the treatment MC-EG. Each image has a scale bar of 250 μm. Fat is observed in red and orange, stained with Nile Red, and proteins and MC fibers are observed in green, stained with fluorescein isothiocyanate, FITC. The samples not subjected to digestion exhibited small cavities (3A1, 3B1, 3C1), originating an irregular network, possibly due to the incorporation of air during comminution and stuffing. The control treatment (FC-3A1) showed a structure with more elongated and continuous protein chains with larger fat globules, when compared with the other samples, with well-defined and delimited borders, as expected for emulsified meat products with good stability. In the treatment FMC (3B1), the structure was spongier and more discontinuous with some coalescing fat globules, with more irregular borders, demonstrating that the chia mucilage may have interfered with the formation of the protein films around the fat globules, thus leading to a lower stability.

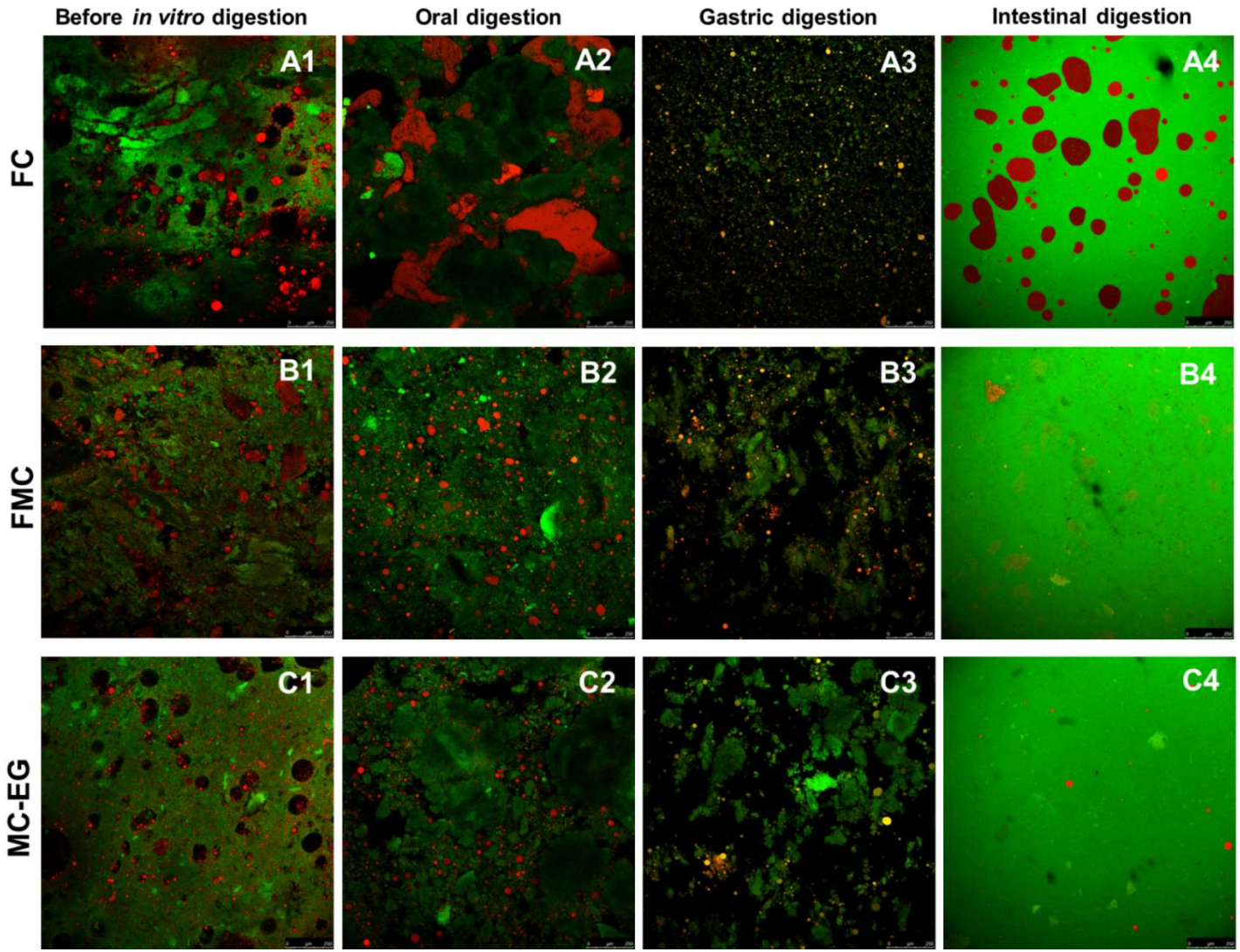

Fig 3. Confocal scanning laser microscopy images of Bologna sausages before in vitro digestion and after oral, gastric and, intestinal phase. For treatments denominations, see Table 1. Magnification: 10 x; Scale bar: 250 µm.

In contrast, the formulation MC-EG (3C1) showed a more compact and uniform organization with smaller and well delimited fat globules. Han, Clausen, Christensen, Vossen, Hecke & Bertram (2018) first evaluated the microstructure of a meat product through Coherent anti-Stokes Raman scattering (CARS) and reported that the protein-fat network was clearly altered with the addition of fiber, and the type of fiber had a strong impact on the extent and size of the dispersed lipid droplets in the protein network.

When the samples were combined with physiological salivary fluids and amylase enzyme (3A2, 3B2, and 3C2), a more swollen, less compact structure was observed, possibly due to the incorporation of sodium ions present in the simulated saliva fluids. The images 3B2 and 3C2 showed larger interstitial spaces (FMC and MC-EG) and the control sample (3A2) showed larger fat aggregates no longer in the form of globules, involved by more disorganized protein chains.

Probably, the gastric digestion provided a more severe and very interesting effect on the microstructure of meat products. For all treatments, a large disintegration of the myofibrillar structure was observed, with a complete disintegration in the control sample FC (3A3). In contrast, the images (3B3 and 3C3) corresponding to the treatments made with MC and emulsion gel showed a distribution of protein aggregates separated by irregular voids, thus demonstrating that the performance of pepsin was not as effective in these samples when compared with the control, corroborating with the results found for the protein digestibility of this study. Baugreet, Gomez, Auty, Kerry, Hamill & Brodkorb (2019) studied the addition of lentil flour and vegetable proteins in restructured meat model systems, and evaluated the microstructural changes of the products during the *in vitro* digestion. The authors observed that the enzymatic mechanism varied among all samples, and, as in this study, the rupture of the food matrix was discontinuous and did not appear to have been affected by pepsin in the same way among the evaluated products. Possibly, both the chia mucilage and the emulsion gel exerted a steric hindrance due to increased viscosity, making it difficult for digestive enzymes to access the active sites of proteins. Lazaro et al. (2018) simulated a micro-digestion process with chia mucilage, and found that this polymer undergoes a slight reduction of viscosity during digestion, thus suggesting that mucilage can withstand acidic environments and enzymatic degradation, retaining its polymeric form.

The images 3A4, 3B4, and 3C4 show the digestion of the samples after the intestinal phase. A complete disintegration of the protein structure was observed for all treatments, with the presence of very fine filaments and some fat fragments, which were more evident in the control treatment FC (3A4), which exhibited a higher amount of fat, with larger globules were initially present in this product. The size of fat globules may influence the rate of lipolysis, once smaller droplets allow a larger interfacial area available for the performance of the digestive lipases (Seimon, Wooster, Otto, Golding, Day, et al., 2009).

4. Conclusion

This study allowed for the first time to correlate the subjective appetite sensations and *in vitro* digestibility analyses, and to assess the microstructural changes of a reformulated meat product made with the chia mucilage in an intragastric environment. In addition, the MC and the MC-based emulsion gel and sodium alginate led to a lower protein digestibility in the Bologna sausages. However, fiber-enriched products provide several other benefits such as delayed glucose absorption, reduced energy value, and increased intestinal mobility.

Healthy volunteers were not able to find differences in perception of satiety after consuming the reformulated Bologna sausages with reduced energy value and saturated fat (reductions up to 30.9% and 44.5%, respectively) made with chia mucilage (MC) and MC-based emulsion gel and sodium alginate as fat substitutes, when compared to a control treatment made with 20% pork back fat. Thus, it is possible to conclude that the fat substitutes of this study have the same satiating power as the conventional source of saturated fat commonly used in commercial formulations.

Future studies are needed to better understand the perceptions of appetite with longer intervention, using samples with higher MC concentrations. Also, this study elucidated the structural changes in meat products made with the addition of emulsion gel and mucilage during a digestive process simulating the physiological conditions, and how the formulation can influence the bioaccessibility of the protein.

Declarations of interest

The authors declare that they have no conflict of interest.

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DISCUSSÃO GERAL

O presente trabalho justifica-se pela recente necessidade de reformular produtos cárneos gerando apelos mais saudáveis em contraponto aos elevados teores de gordura que estes, em sua maioria, apresentam. A contundente associação entre altos níveis de ingestão de gordura, particularmente, gordura saturada e algumas doenças crônicas tais como, hipertensão e doenças cardiovasculares, resulta em campanhas muito negativas referentes ao consumo de produtos cárneos, principalmente, produtos emulsionados tais como mortadelas, contendo em geral de 20-30% de teor lipídico.

No entanto, a substituição parcial ou total de gordura saturada, geralmente incorporada em produtos cárneos na forma de toucinho, apresenta muitos problemas tecnológicos a serem superados, relacionados à estabilidade física, química e sensorial promovida por esse componente cárneo. Assim, a busca de substitutos requer uma criteriosa seleção de ingredientes com avaliação de suas propriedades globais que poderão influenciar os parâmetros de identidade e qualidade dos respectivos produtos cárneos tradicionais. Atualmente, há uma tendência crescente de introdução de ingredientes funcionais mais saudáveis como fibras, proteínas não-cárneas e óleos com perfis de ácidos graxos mais favoráveis em inúmeras investigações científicas, além de uma busca incessante por novos compostos que possam minimizar os impactos causados pela ausência das gorduras e, que, possam ainda trazer benefícios para a saúde. Soma-se a estes desafios inerentes da reformulação lipídica em produtos cárneos, a necessidade de redução de aditivos nesses produtos, outra tendência atual e desafiadora, porém muito desejada pelos consumidores, comumente chamada de “clean label”.

A mucilagem de chia caracterizou-se como um ingrediente funcional com perspectivas muito promissoras, tanto para substituição da gordura animal como para redução de fosfatos nos produtos cárneos emulsionados, com alta capacidade de retenção de água e formando dispersões viscosas mesmo em baixas concentrações. Neste contexto, os objetivos deste estudo foram estudar a mucilagem de chia em suas diferentes formas de aplicação sobre as propriedades tecnológicas, sensoriais, nutricionais, microestruturais e fisiológicas em produtos cárneos emulsionados com redução de gorduras e fosfatos.

Na primeira etapa do estudo (capítulo 2) foi realizada uma caracterização da mucilagem de chia (MC) e uma avaliação das suas propriedades reológicas em diferentes

concentrações de dispersões aquosas ou géis de mucilagem. As propriedades tecnológicas dos sistemas modelos cárneos com as dispersões de mucilagem também foram avaliadas. O comportamento reológico da MC foi viscoelástico com uma estrutura de gel, com características de gel fraco na frequência estudada. Além disso, a MC apresentou propriedades mecânicas estáveis após ser submetidas a diversas oscilações de temperatura. Nos sistemas modelos cárneos, a MC demonstrou seu grande potencial como agente de alta capacidade de retenção de água, uma vez que diminuiu a exsudação dos sistemas modelo cárneos emulsionados fatiados bem como melhorou a estabilidade da emulsão cárnea. As diversas concentrações das dispersões ou géis de mucilagem não tiveram influência significativa nas características do produto, contudo, as maiores concentrações adicionadas (5%) interferiram nas propriedades de textura e cor das amostras. Apesar disso, este estudo inicial demonstrou preliminarmente o potencial da mucilagem de chia como ingrediente substituto de gordura em produtos cárneos.

Na etapa seguinte do trabalho, (capítulo 3) a mucilagem de chia foi investigada como substituto de gordura e também como ingrediente alternativo à redução ou ausência de fosfatos, adicionada de duas formas diferentes (em pó ou em gel) em mortadelas. Os resultados mostraram que a adição de 2% de mucilagem de chia na forma de gel, na ausência total de fosfatos e com redução de 50% destes aditivos, apresentaram a mesma aceitação e parâmetros textura de elasticidade e coesividade semelhantes ao tratamento controle. A adição de mucilagem de chia, independente da concentração final e forma de incorporação afetou os parâmetros de cor, com diferenças consideráveis ao se comparar com o controle. A partir dos resultados obtidos, observou-se que a forma de gel foi a que produziu melhor estabilidade e parâmetros de textura mais próximos ao tratamento controle, até o nível de 2% de adição. Contudo, nos níveis de 4% de mucilagem, houve maior efeito sobre as propriedades microestruturais com aparecimento de uma estrutura proteica menos coesa e uniforme. As análises de ressonância magnética nuclear de baixo campo sugeriram uma mobilidade de água mais restrita nos tratamentos com 4% de mucilagem de chia, independente da presença ou não dos fosfatos. Esta etapa do trabalho demonstrou, de forma inédita, a ação da mucilagem como alternativa ao uso de fosfatos em mortadelas com redução de gordura, com perspectivas muito promissoras.

O capítulo 4 explorou a utilização da mucilagem de chia como um agente gelificante em emulsões géis com azeite de oliva e seu potencial para uso como substituto de

gordura em produtos cárneos emulsionados. Nesta parte do trabalho, a mucilagem de chia foi avaliada isoladamente e, em combinação com outros agentes gelificantes. A mucilagem mostrou uma boa capacidade de emulsificação e formação de gel, com uma maior estabilidade quando associada com a proteína do soro de leite. Observou-se também uma compatibilidade entre proteína de soro de leite, colágeno, alginato e mucilagem, formando um sistema de emulsão gelificado com azeite de oliva, com notável potencial tecnológico para a reformulação de produtos cárneos emulsionados.

O estudo conduzido no capítulo 5 foi uma continuidade desta parte do estudo com emulsões géis. Foi realizada uma seleção das melhores formulações de emulsões géis com base no desempenho tecnológico e reológico, para aplicação como substitutos de gordura em mortadelas. As emulsões géis contendo a MC, colágeno, alginato de sódio e proteína do soro de leite foram avaliadas. A substituição total da gordura suína pelas emulsões géis com mucilagem de chia e azeite de oliva, assim como a redução de 50% no teor de fosfato, foram consideradas exequíveis do ponto de vista tecnológico e recomendada do ponto de vista nutricional. Foi possível obter produtos mais saudáveis com características de qualidade comparáveis a uma formulação controle, com alta estabilidade, aceitação sensorial e melhor perfil de ácidos graxos.

Após a obtenção de diversas respostas tecnológicas importantes para este projeto com mucilagem de chia como novo ingrediente para produtos cárneos mais saudáveis, o capítulo 6 teve por objetivo investigar as propriedades de saciedade deste ingrediente funcional quando aplicado em produto cárneo emulsionado. Sendo a mucilagem de chia considerada uma fibra solúvel, diversos estudos relatam a propriedade de formação de soluções viscosas no estômago que proporcionam maior sensação de plenitude e retardam o esvaziamento gástrico. O Artigo 6, portanto, avaliou os efeitos da substituição da gordura pela mucilagem de chia e por uma emulsão gel a base de mucilagem em mortadelas sobre as percepções de saciedade em indivíduos saudáveis e investigou as mudanças microestruturais que ocorrem durante uma simulação de digestão *in vitro.* Voluntários saudáveis não foram capazes de expressar diferenças em relação à percepção de saciedade quando ingeriram mortadelas reformuladas com redução de valor calórico e teor de gordura saturada (reduções de até 30.9% e 44.5% respectivamente) adicionadas de mucilagem de chia e de emulsão gel quando comparadas com um tratamento controle com 20% toucinho. A simulação da digestão *in vitro* revelou um comportamento muito interessante das amostras com mucilagem e forneceu fortes evidências

de que a atuação da pepsina não foi tão eficaz nestas amostras ao se comparar com o tratamento controle. Nas amostras com mucilagem de chia e emulsão gel, as imagens de microestrutura mostraram uma distribuição de agregados de proteínas separados por espaços vazios irregulares, enquanto na amostra controle houve desintegração completa, o que corroborou diretamente com os menores valores encontrados para a digestibilidade proteica dos tratamentos com mucilagem. De forma inédita, foi possível correlacionar resultados de uma avaliação subjetiva de sensações de apetite com análises objetivas de simulação de digestibilidade *in vitro*, juntamente com a investigação das mudanças microestruturais que ocorrem em um produto cárneo reformulado com a mucilagem de chia, em um ambiente intragástrico.

CONCLUSÃO GERAL

Este estudo demonstrou a viabilidade tecnológica da mucilagem de chia como um inovador ingrediente para substituição de gordura e redução de fosfato em produtos cárneos emulsionados através da obtenção de dados inéditos que incluíram aspectos tecnológicos, físico-químicos, sensoriais e fisiológicos.

A caracterização realizada da mucilagem de chia (MC) evidenciou que as concentrações nos géis elaborados influenciam as propriedades reológicas, com maiores valores de módulo elástico e viscoso com o aumento das concentrações dos géis. Além disso, os géis de MC preservam suas propriedades mecânicas quando são simuladas as mudanças que ocorrem durante o processamento de produtos cárneos emulsionados, informação muito útil para posterior aplicação tecnológica. Os sistemas modelo cárneos avaliados não tiveram seus parâmetros de qualidade influenciados pelas concentrações dos géis, sendo mais importante a concentração de MC nos produtos. Houve melhorias significativas na estabilidade das emulsões cárneas e diminuição da exsudação na operação de fatiamento, parâmetros de qualidade relevantes para a tecnologia e comercialização de produtos. No entanto, a concentração de MC no produto final teve uma influência significativa na textura e cor, reportando a necessidade de otimização das formulações industriais a serem processadas com tais ingredientes como substitutos de gordura.

Ao se avaliar a redução de fosfatos em mortadelas com teor de gordura reduzido, a adição de até 2% de mucilagem de chia gel (MCG) foi eficaz, e contribuiu para propriedades

de textura semelhantes ao controle e estabilidade. Além disso, apresentou um desempenho sensorial satisfatório, com aceitação global comparável ao tratamento controle, porém o atributo de cor obteve notas menores sendo, como já descrito anteriormente, uma barreira tecnológica a ser superada. A análise TDS permitiu uma melhor caracterização sensorial e entendimento das percepções dos provadores no consumo de produtos cárneos reformulados com redução de gordura e fosfatos e foi coerente com as outras avaliações sensoriais realizadas. A microscopia confocal demonstrou ser uma ferramenta de estudo importante para compreender melhor as alterações nas estruturas proteicas das amostras, e, evidenciou que a MC exerce uma influência nas ligações proteína-proteína e proteína-gordura levando à formação de uma rede proteica mais fracamente ligada e com gotículas de gordura menos entremeadas na matriz. As análises de RMN de baixo campo revelaram um pronunciado efeito da MC na mobilidade e distribuição de água, principalmente nos tratamentos com 4% de MCG. Nestes tratamentos, houve considerável diminuição dos tempos de relaxação e demonstraram que os prótons das amostras tiveram mobilidade mais restrita, o que confirma a grande capacidade de retenção de água da mucilagem.

A elaboração de emulsões géis (EGs) com MC e azeite de oliva foi uma alternativa tecnológica muito promissora para substituição de gordura e redução de fosfatos, uma vez que produtos elaborados com total substituição da gordura suína pelas EGs tiveram desempenho tecnológico semelhante ao tratamento controle, com alta estabilidade, aceitação sensorial, e melhor perfil de ácidos graxos. Observou-se também uma compatibilidade entre proteína de soro de leite, colágeno, alginato e MC, formando um sistema de emulsão gelificado com azeite de oliva, com notável potencial tecnológico para a reformulação de produtos cárneos emulsionados.

Além disso, este estudo permitiu pela primeira vez, que uma avaliação subjetiva de sensações de apetite pudesse ser relacionada com análises objetivas de simulação de digestibilidade *in vitro*, juntamente com a investigação das mudanças microestruturais que ocorrem em um produto cárneo reformulado, com a mucilagem de chia, em um ambiente intragástrico. Os resultados de percepções de saciedade realizada com os voluntários saudáveis não reportaram maiores sensações de saciedade nas amostras com MC e emulsão gel, embora tais amostras apresentassem valor calórico reduzido em relação ao controle, demonstrando que mesmo com um decréscimo do valor energético foi possível propiciar o mesmo poder saciante de uma mortadela com alto valor calórico. Além disso, esta etapa do estudo forneceu uma maior

elucidação das mudanças estruturais que ocorrem com os produtos cárneos adicionados de emulsão gel e mucilagem durante um processo digestivo que simula condições fisiológicas e como a bioacessibilidade da proteína pode ser influenciada por reformulações.

A mucilagem de chia, como conclusão geral, apresentou um grande potencial para ser utilizada como um ingrediente funcional inovador em produtos cárneos, confirmando suas habilidades de formação de gel, estabilização de emulsão e propriedades nutricionais. Estudos futuros que visem a avaliação da estabilidade oxidativa destes novos produtos reformulados são essenciais para a complementação deste trabalho. Este estudo aponta para novos direcionamentos e uma grande junção de áreas como Tecnologia e Nutrição, que devem ser cada vez mais inter-relacionadas para que importantes mecanismos físico-químicos e fisiológicos sejam explicados para uma consistente e factível reformulação de produtos cárneos que atendam aos anseios globais dos consumidores e contribuam para a melhoria da saúde.

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ANEXO 1. Aprovação do projeto de pesquisa pelo Comitê de ética em pesquisa da Faculdade de Ciências Médicas da Universidade Estadual de Campinas – UNICAMP.

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeitos da adição de fibras solúveis em produtos cárneos sobre as propriedades físico-químicas, funcionais, sensoriais e saciedade

Pesquisador: ANA KAROLINE FERREIRA IGNACIO CAMARA

Área Temática:

Versão: 1

CAAE: 68151217.8.0000.5404

Instituição Proponente: Faculdade de Engenharia de Alimentos

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.085.153

Apresentação do Projeto:

A obesidade está intimamente ligada a diversas doenças crônicas, incluindo as doenças cardiovasculares constituindo-se num dos principais problemas de saúde da atualidade. A associação com a dieta e os aspectos relacionados à composição de produtos cárneos, em geral, caracterizados por elevados teores de sódio, gordura, colesterol e ausência de fibras resultam na busca de alternativas para sua reformulação e promoção de apelos mais saudáveis. Produtos cárneos apresentam características de grande capacidade de saciedade e, a incorporação de compostos bioativos como por exemplo as fibras solúveis, podem contribuir ainda mais para maior sensação de plenitude e redução do apetite. A chia é um importante alimento funcional, rica em ácidos graxos ômega-3, proteínas e fibras dietéticas. Além disso, na presença de água a chia exsuda uma fibra solúvel (mucilagem). Esta fração solúvel é de particular interesse devido às suas propriedades funcionais e nutricionais e podem ser excelentes ingredientes para substituição da gordura em alimentos. Mortadelas são produtos cárneos amplamente consumidos no Brasil e possuem de modo geral altos teores de gorduras saturadas e sódio.Este projeto terá como objetivo estudar os efeitos da adição da mucilagem de chia como substituto parcial de gordura suína sobre as propriedades funcionais, características físico-químicas, sensoriais e percepção da saciedade em produtos cárneos emulsionados, sendo dividido em três etapas. Na primeira etapa

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Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CAMPINAS, 26 de Maio de 2017

Assinado por:
Renata Maria dos Santos Celeghini
(Coordenador)