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Natural antioxidants and lipid profile improvement in lamb meat products

Antioxidantes naturais e melhoria do perfil lipídico em produtos à base de carne de cordeiro

Pirassununga
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Tese apresentada à Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo, como parte dos requisitos para a obtenção do título de Doutor em Ciências do programa de Pós-graduação em Engenharia de Alimentos.

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I dedicate this work to my mother, Carmelita Leandro de Oliveira and my grandfather, Edésio Leandro *in memorian*. Reasons abound.

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ABSTRACT

CARVALHO, F. A. L. **Natural antioxidants and lipid profile improvement in lamb meat products**. 2020. 139 f. Ph.D. Thesis – Faculty of Animal Science and Food Engineering, University of São Paulo, Pirassununga/SP, Brazil, 2020.

This study evaluated the effect of reformulation (fat substitution and use of natural antioxidants) on lamb meat products during refrigerated storage: (i) lamb burger with enhanced lipid profile and extracts of guarana seed and pitanga leaf as natural antioxidants; (ii) cooked animal fat-substituted sausages with flaxseed, olive and chia oils; and (iii) fresh lamb sausage with improved lipid profile and different levels of turmeric extract. Physico-chemical, sensory and oxidative stability characteristics were evaluated during their shelf life. Analyzes included proximate composition (moisture, protein, fat and ash) and sensory acceptance (day 0); pH, color (L *, a *, b *), TBARs, carbonyl content and visual sensory evaluation (0, 6, 12 and 18 days for burgers and fresh sausages and 0, 30, 60 and 90 for cooked sausage); fatty acids and volatile compounds profile (0 and 18 days in burgers and 0 days in cooked sausages), and DPPH (0, 6, 12 and 18 days in burgers and fresh sausages). The guarara seed and pitanga leaf extracts showed higher antioxidant activity to protect of hamburgers against lipid and protein oxidation during the storage time than the synthetic antioxidant, without compromising the chemical and sensory characteristics of lamb hamburger. Vegetable oils provided consistent improvements in fatty acid profile and nutritional indexes of cooked lamb sausages; mainly chia and flaxseed. However, sausages made with chia oil showed a loss in texture parameters and sensory analysis. On the other hand, flaxseed oil was able to improve the lipid profile of sausages without changing its technological and sensory characteristics. Turmeric extract showed higher antioxidant capacity during storage and, consequently, promoted lower lipid oxidation, confirmed by the results of TBARs and lower formation of volatile compounds derived from oxidation, even at a lower dose than synthetic antioxidant. Thus, guarana seed extracts and pitanga leaves can replace the synthetic antioxidant in lamb burgers without causing detrimental changes in their physicochemical and sensory properties. Among the oils tested, flaxseed oil is most recommended as a fat substitute in cooked lamb sausages. Turmeric Extract is a

promising natural antioxidant to extend the shelf life of fresh lamb sausages with improved lipid profile without compromising its quality characteristics.

Keywords: antioxidant activity, fat replacement, lipid and protein oxidation, natural antioxidante, sensorial analysis, volatile compounds

RESUMO

CARVALHO, F. A. L. **Antioxidantes naturais e melhoria do perfil lipídico em produtos à base de carne de cordeiro**. 2020. 139 f. Faculdade de Zootecnia e Engenharia de Alimentos, Pirassununga/SP, Brasil, 2020.

Este estudo avaliou o efeito da reformulação (substituição de gordura e uso de antioxidantes naturais) em produtos à base de carne de cordeiro durante armazenamento refrigerado: (i) hambúrguer de cordeiro com perfil lipídico melhorado e extratos da semente de guaraná e folha de pitanga como antioxidantes naturais; (ii) salsichas cozidas com substituição de gordura animal por óleos de linhaça, oliva e chia e (iii) linguiça frescal de cordeiro com perfil lipídico melhorado e diferentes níveis de extrato de cúrcuma. As características fisico-químicas, sensoriais e estabilidade oxidativa foram avaliadas durante sua vida de prateleira. As análises incluiram a composição centesimal (umidade, proteína, gordura e cinzas) e aceitação sensorial (dia 0); pH, cor (L *, a *, b *), TBARs, grupos carbonilas e avaliação sensorial visual (0, 6, 12 e 18 dias nos hambúrgueres e nas linguiças frescas e 0, 30, 60 e 90 para salsicha cozida); perfil de ácidos graxos e compostos voláteis (0 e 18 dias nos hambúrgueres e 0 dia nas salsichas cozidas), além de DPPH (0, 6, 12 e 18 dias nos hambúrgueres e nas linguiças frescas). Os extratos da semente de guarará e da folha de pitanga apresentaram maior atividade antioxidante na proteção dos hambúrgueres contra oxidação de lipídios e proteínas durante o tempo de armazenamento que o antioxidante sintético, sem comprometer as características físico-químicas e sensoriais do hambúguer de cordeiro. Óleos vegetais proporcionaram melhorias consistentes no perfil de ácidos graxos e nos índices nutricionais das salsichas de cordeiro cozidas; principalemente os de chia e linhaça. No entanto, salsichas elaboradas com óleo de chia apresentaram alterações indesejáveis nos parâmetros de textura e na analise sensorial. Por outro lado, o óleo de linhaça foi capaz de melhorar o perfil lipídico das salsichas sem alterar suas características tecnológicas e sensoriais. O extrato de cúrcuma apresentou maior capacidade antioxidante durante o período de armazenamento e, consequentemente, promoveu menor oxidação lipídica, confirmada pelos resultados de TBARs e menor formação de compostos voláteis derivados da oxidação, mesmo em dose inferior à do antioxidante sintético.

Assim, os extratos de sementes de guaraná e folhas de pitanga podem substituir o antioxidante sintético em hambúrgueres de cordeiro, sem causar alterações prejudiciais em suas propriedades físico-químicas e sensoriais. Entre os óleos testados, o óleo de linhaça é mais recomendado como substituto da gordura animal em salsichas de cordeiro cozidas. O extrato de cúrcuma é um antioxidante natural promissor para prolongar a vida de prateleira de linguiça fresca de cordeiro com perfil lipídico melhorado sem comprometer suas características de qualidade.

Palavras-chave: atividade antioxidante, antioxidante natural, análise sensorial, compostos voláteis, oxidação lipídica e proteica, substituição de gordura.

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SUMMARY

1. GENERAL INTRODUCTION

Food reformulation is a relatively new strategy aimed at developing foods with properties beneficial to human health. In this sense, it plays an important role from a nutritional point of view, and can provide significant population benefits. Food reformulation is the redesign of the recipe for an existing processed food aimed primarily at improving its nutritional profile. Traditionally, salt, sugar and saturated and trans fats are the constituents considered harmful to human health and the goal of food reformulation to reduce and/or replace them. Although, reformulation in food processing is not limited to this.

Several studies have evaluated the replacement of animal fat added to meat products with different sources of vegetable oils (BARROS et al., 2020; PINTADO et al., 2017; PIRES et al., 2019). The challenge is to find an oil capable of enhancing the fatty acid profile without affecting the characteristics of the final product. Vegetable oils such as chia, olive, linseed and tiger nut oils can improve the fatty acid profile due to their higher amount of unsaturated fatty acids characterized by their health benefits (BARROS et al., 2020; DOMÍNGUEZ et al., 2017a). However, the use of oils rich in unsaturated fatty acids may compromise the shelf life of the product due to increased lipid oxidation.

To prevent or delay the oxidation reactions of meat and meat products, the industry adds mainly synthetic antioxidants (such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG)) during processing (CUNHA et al., 2018). However, some studies have shown the adverse effects of synthetic antioxidants on consumer health, and health organizations do not recommend their use due to the toxicity and carcinogenicity problem (Abraham et al. 1986; Ahmad et al. 1995; Faine et al. 2006), which increased the demand for natural antioxidants. Thus, antioxidants are another constituent of the food recipe subject to reformulation. For this reason, this theme has received the attention of several researchers aiming to find a natural antioxidant capable of replacing synthetic antioxidants without compromising product quality and maintaining or extending their shelf life.

Natural antioxidants are obtained from sources such as plant materials and their extracts, herbs, spices and essential oils; their effectiveness depends mainly on the composition of the plant extract and its antioxidant activity. Several studies have been conducted to identify part of plants from which antioxidants can be extracted and used as a substitute for synthetic antioxidant in meat products (AGREGÁN et al., 2018; FERNANDES et al., 2016; MANCINI et al., 2015). Pitanga leaves (Eugenia uniflora L.) and guarana seeds (Paullinia cupana) contain considerable amount of phenolic compounds, which have recognized antioxidant properties (LORENZO et al., 2018; PATEIRO et al., 2018). Similarly, curcuma longa L. (turmeric) is a widely used herbaceous plant for its flavor, color and preservative properties that has been studied for its high antioxidant capacity (MANCINI et al., 2015).

High lipid and protein content is a major contributor to oxidative and microbial damage to meat and meat products (PATEIRO et al., 2018). Among red meat, lamb stands out for containing approximately half of unsaturated fatty acids. The higher amount of unsaturated fatty acids enables the oxidative processes of meat (KUMAR et al., 2016). When used to produce products with improved lipid profile by replacing animal fat with vegetable oils, the need for antioxidants is even more evident.

2. OBJETIVES

General objective

To evaluate the effect of adding vegetable oils and natural antioxidants on physicochemical, sensory characteristics, oxidative stability and lipid profile of lamb meat products.

Especific objetives

- To evaluate the effect of guarana seed and pitanga leaf extracts as natural antioxidants in fat-replaced lamb burger during cold storage (2ºC);

- To evaluate the effect of replacing animal fat with vegetable oils (chia, flaxseed and olive) on cooked lamb sausages during cold storage (2ºC);

- To evaluate the effect of different levels of turmeric extract as a natural antioxidant on fresh lamb sausage and to recommend the best inclusion level.

3. REVIEW OF LITERATURE

The occurrence of chronic diseases related to diets and obesity is a problem that affects large numbers of people around the world and across income ranges. Along with the food industry's focus on high efficiency, palatability and convenience, a food system was developed with diets full of high caloric products, with high sodium content and nutritionally unbalanced (BRANCA et al., 2019). In addition, some substances frequently used to prevent oxidation and prolong the shelf life of these foods, have their use not recommended because it presents adverse health effects if consumed frequently (FALOWO; FAYEMI; MUCHENJE, 2014; HASHEMI et al., 2017).

On the other hand, the term "processed"; on food, has nowadays often been associated with something negative, unhealthy. However, food processing is fundamental, and transforms raw agribusiness products into safe and edible, in addition to extending the shelf life and makes it possible to produce food that is nutritionally balanced and beneficial to health (RAIKOS e RANAWANA, 2019). It is worth highlighting the responsibility of consumers to make balanced choices based on information and the direction of the food industry to meet these demands.

The reformulation of food comprises the actions of the industry to redesign an existing product, aiming to improve its nutritional profile. This action has wider consequences when applied to popular and affordable products. In this way, to improve the quality of the lipid fraction of meat products as well as the replacement of certain substances with questionable or not recommended use by natural substances by means of reformulation, can help improve the overall quality of the diet, enabling healthier choices for individuals and achieving population-wide results (MACDIARMID et al., 2018). These products are widespread and with high consumption around the world.

Traditionally, the reformulation of meat products aims at reducing or replacing constituents such as salt, fat (saturated, trans) and cholesterol. The focus of this work, however, is directed both to the substitution of animal fat and to the use of natural antioxidants in products with improved lipid profile and their effects on the characteristics of the products (physical-chemical, sensory and shelf life).

The main concern with the effect of the diet on the health of consumers observed in recent years, resulted in an increase in demand for healthier products. Fat, together with cholesterol, are the components that most worry consumers of processed meat products. In this sense, it became necessary to know strategies to improve nutritional quality without compromising the typical characteristics of each product as well as its acceptability.

Several studies have pursued this goal by replacing animal fat with vegetable oils (BARROS et al., 2020; DOMÍNGUEZ et al., 2017a; LORENZO et al., 2017; PINTADO et al., 2017; PIRES et al., 2019). However, several factors may influence this strategy, such as the type of oil, the way it is inserted (directly, emulsified, structured gel, etc.) and susceptibility to oxidation. In addition, the addition of fat to meat products, as well as other ingredients, serves a purpose, contributing to the flavor, consistency and texture. Vegetable oils have lower fat globules when compared to animal fat and this provides a higher protein-protein and protein-lipid interaction providing greater compression strength (YOUSSEF; BARBUT, 2009). In addition, the different composition and consequently different color shades of vegetable oils may affect the color of the product and its acceptability.

Fatty acid intake has recommendations for a lower proportion of saturated fatty acids (SFAs) and a higher proportion of monounsaturated fatty acids (MUFAs) or polyunsaturated fatty acids (PUFAs), a better PUFA / SFA and n-6 / n-3 ratios and lower values for thrombogenicity (TI) and atherogenicity (AI) indices. (ULBRICHT; SOUTHGATE, 1991). FAO (2010) recommends a PUFA / SFA ratio above 0.85 and that the n-6 / n-3 ratio should not exceed the upper limit of 4.00.

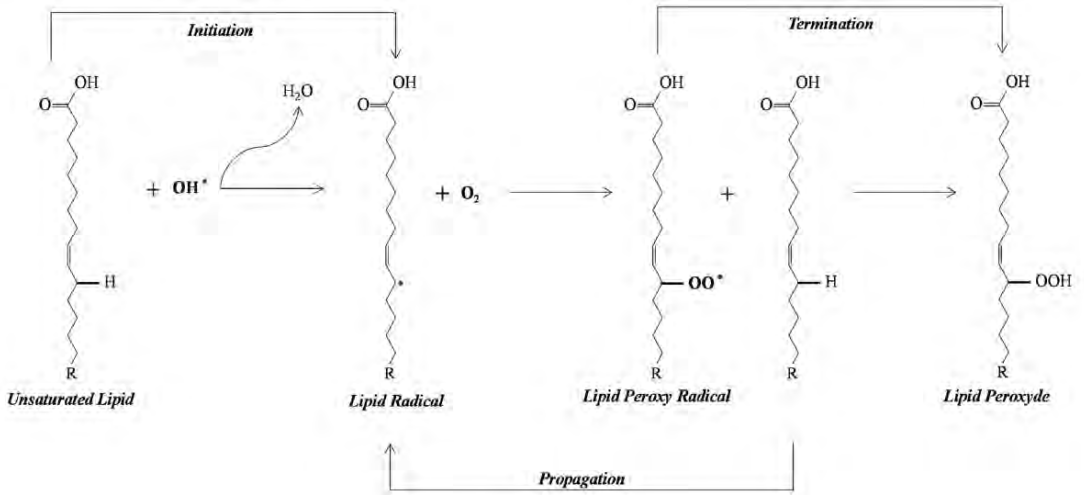
Olive oil has a low saturated (<23%) and high unsaturated fatty acids contents in its composition (>71%). In addition, oleic acid, which is the majority in olive oil (>63%) (Gavahian et al., 2019), has beneficial properties for human health associated with reduced risk of cardiovascular disease (WHO, 2003). Similarly, chia, linseed and tiger nut oils, because they contain better PUFA / SFA ratio, are also candidates for animal fat substitutes with health benefits. (BARROS et al., 2020; DOMÍNGUEZ et al., 2017b). The challenge is to find a vegetable oil capable of enhancing the fatty acid profile without affecting the physical and chemical properties of the product due to its

substantially different characteristics such as taste, color and consistency, and sensory as unsaturated fatty acids are oxidized faster, which can lead to rancidity.

Oxidative processes are one of the main causes of meat and meat products deterioration. They occur throughout the productive chain, acting from the conversion of muscle into meat, following the processing, storage and even during preparation (CUNHA et al., 2018). Oxidative reactions begin at the post-slaughter stage during muscle-meat conversion as a result of imbalance in the pro-oxidative and antioxidant system *in vivo* (KUMAR et al., 2016). These reactions lead to undesirable changes such as discoloration, development of foreign taste and odour, change of texture, loss of nutritional quality, thereby limiting the shelf life of the product, in addition to the formation of secondary compounds possibly harmful to human health.

Several intrinsic and extrinsic factors influence the oxidative stability of the meat, among them are the pH, temperature, concentration of prooxidant agents, but above all the composition of the lipid and protein fraction. High fat and protein contents make meat products more susceptible to oxidative and microbial damage (PATEIRO et al., 2018). Lipid oxidation occurs in three different phases (Fig. 1.), called initiation, propagation and termination in which oxygen promotes the degradation of fatty acids through free radicals. In the initiation phase there is the abstraction of a hydrogen radical from an unsaturated fatty acid, generating an alkyl radical (R*) that reacts with oxygen, forming peroxide radicals (ROO*) which in turn react with unsaturated fatty acids in the propagation phase and produce hydroperoxides (ROOH), the main lipid oxidation compounds (CUNHA et al., 2018). These primary compounds do not affect the characteristics of the products; however, they are unstable and tend to react in free radical chain forming aldehydes, ketones, alkanes and other secondary compounds of lipid oxidation. Ketones and short chain aldehydes, among others, affect the overall acceptability of meat products (LORENZO et al., 2018).

Figure 1. Phases of lipid oxidation of unsaturated fatty acids.



(CUNHA et al., 2018).

In addition, protein oxidation is related to lipid oxidation. Protein carbonyls are the main products of protein oxidation, and the carbonyl radical is the indicator of this oxidation. The major pathways of protein carbonylation include: direct oxidation of amino acid side chains (ESTÉVEZ et al., 2011); and binding of the amino acid side chains of the protein to non-protein carbonyl compounds derived from lipid peroxidation (REFSGAARD; TSAI; STADTMAN, 2000). LUND et al. (2011) described a correlation between protein carbonylation and protein functionalities, including water retention capacity, texture, and nutritional value. ESTÉVEZ et al. (2011) associated the intensity of protein carbonylation with decreasing water-binding ability of the meat proteins.

Many researchers have studied the use of natural plant-derived antioxidants such as fruits, plants and oils and their effects on lipid and protein oxidative stability and sensory attributes. Their effectiveness, however, depends on factors such as concentration of antioxidant agents, form of extraction and application, processing and so on. Most studies on natural antioxidants were conducted with meat and meat products from pigs or cattle, but only few studies with natural antioxidants in lamb meat

products. The lipid composition of meat varies with animal species and lamb meat has approximately half of unsaturated fatty acids (D'ALESSANDRO et al., 2019). From a nutritional point of view, this is a positive point, however, the higher amount of unsaturated fatty acids favors the oxidation of meat. Additionally, lamb meat is classified as red meat due to its high concentration of myoglobin, which also predisposes to oxidation. In fact, reformulating lamb meat products may be considered an opportunity to provide consumers with healthier, more nutritious and sustainable foods.

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CHAPTER I - Effect of guarana (Paullinia cupana) seed and pitanga (Eugenia uniflora L.) leaf extracts on lamb burgers with fat replacement by chia oil emulsion during shelf life storage at 2°C*

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Effect of guarana (*Paullinia cupana*) seed and pitanga (*Eugenia uniflora* L.) leaf extracts on lamb burgers with fat replacement by chia oil emulsion during shelf life storage at 2 °C

Abstract

The effects of guarana seed and pitanga leaf extracts on the physical–chemical and sensory characteristics, and oxidative stability of modified atmosphere-packaged lamb patties with fat replacement during storage (2 °C) were investigated. Four treatments were prepared: control (without antioxidant); with BHT (10 mg/kg); with 250 mg/kg guarana extract (G250); with 250 mg/kg pitanga extract (P250). Analysis included the proximate composition (moisture, protein, fat, and ash) and sensory acceptance (day 0); pH, color (*L**, *a**, *b**), TBARs, carbonyl content, DPPH, and visual sensory assessment (0, 6, 12, and 18 days); fatty acid profile and volatile compounds (0 and 18 days). G250 and P250 did not alter the centesimal composition and the acceptance of the lamb burgers on day 0. The extracts also delayed discoloration of the burgers, endowed the reddest intensity, and retarded lipid and protein oxidation throughout storage time, particularly P250, which presented the lowest TBARs levels (6.92 mg MDA/kg) and carbonyl values (5.59 nmol carbonyl/mg), and the highest antioxidant activity (249.48 µg Trolox/g), at day 18. The MUFA, SFA, and PUFA levels, AI, TI, and h/H ratio were comparable between treatments; only the n-6/n-3 ratio was higher in P250 treatment but within the recommended levels. More volatile compounds were derived from lipid oxidation in the control and BHT treatments than G250 and P250 treatments. As a result, both G250 and P250 groups are effective against color deterioration, and lipid and protein oxidation, without impairing the sensorial characteristics, representing a promising alternative to replace synthetic antioxidants by natural products in lamb burger.

Keywords*:* Antioxidant activity, Natural antioxidant, *Paullinia cupana*, *Eugenia uniflora* L., lipid and protein oxidation, sensorial analysis

1. INTRODUCTION

The quality of meat and meat products is influenced by many factors throughout its shelf life (temperature, presence of oxygen and light, microbial activity). These factors may influence consumers' acceptance of these products, as they alter the color, flavor, nutritional value, and other quality aspects of the meat (Lorenzo, Batlle, & Gómez, 2014a). One of the main causes of meat deterioration is oxidation (lipid and protein). Lipid oxidation may cause other undesirable effects, such as loss of essential fatty acids, taste, and discoloration, leading to changes in organoleptic properties (Zamuz et al., 2018). On the other hand, lipid reformulation by replacing a portion of the animal fat by fat substitutes containing n-3 PUFA-rich oils may provide healthier characteristics to the food product, thus meeting the demands of health-conscious consumers (Agregán et al., 2018; Dominguez et al., 2017). In this regard, the application of chia in meat products is recently increasing. Low-fat burgers were produced with chia oil microparticles substituting 50% of fat. The sensory quality suffered whereas the hardness was not affected, but the cooking loss and fat retention was improved (Heck et al., 2017). Moreover, the chia oil did not have a major impact on the volatiles profile of raw and cooked burgers for both the unencapsulated and microencapsulated forms (Heck et al., 2019).

To avoid or retard oxidation reactions, synthetic antioxidants, notably, butylated hydroxytoluene (BHT), are frequently used during processing. However, in recent years there has been a demand for natural, healthier products (dos Santos et al., 2016; Lorenzo et al., 2016). This interest extends to producing meat products with new health attributes (Dominguez, Pateiro, Agregán, & Lorenzo, 2017a), as well as the use of natural antioxidants from vegetable extracts that may be an alternative to synthetic food additives in oxidation retardation, and color and texture stabilization (Fernandes et al., 2016a).

Several studies have evaluated the use of plant extracts or seeds as antioxidants in sheep meat and meat products (Fernandes et al., 2017, 2018; Munekata, Franco, Trindade, & Lorenzo, 2016). Leaves of pitanga (*Eugenia uniflora* L.) contain compounds that confer a range of human health benefits, for instance, prevention of hypertension, yellow fever, rheumatism, and digestive disorders of stomach diseases (Martinez-Correa et al., 2011). As a result, leaf extracts of pitanga

have been studied as an antioxidant additive in meat and meat products (Lorenzo et al., 2018; Vargas, Pereira, da Costa, de Melo, & do Amaral Sobral, 2016). Similarly, guarana (*Paullinia cupana*) seed is abundant in phenolic compounds that are responsible for its antimicrobial and antioxidant activities (Pateiro et al., 2018). Thus, this study evaluated the effect of the guarana seed and pitanga leaf extracts, as natural antioxidants, on the physical–chemical and sensory characteristics, as well as protein and lipid oxidation, of modified atmosphere-packaged lamb patties with animal fat substitution by chia oil during storage at 2 °C.

2. MATERIAL AND METHODS

2.1. Plant material and extract preparation

Ground guarana seeds were supplied by Florien (Piracicaba, São Paulo, Brazil), whereas red pitanga leaves were harvested in Pirassununga (São Paulo, Brazil), rinsed with running distilled, water sanitized with sodium hypochlorite solution (2.5%) for 15 min, and dried using a force air-circulating oven (MA035/5, Marconi, Brazil) at 40 °C for 72 h. This material was ground in a thermostated rotor mill (MA-090CFT, Marconi, Brazil) and sieved (48 mesh) for particle size uniformity. Plant material (1 g) was dispersed in 10 mL hydroethanolic solvent (40:60, water:ethanol) and this solution remained 45 min in an ultrasound-assisted extraction bath (MaxiClean 1400A, Unique, Brazil) at room temperature, under darkness conditions. Then, the extraction process continued in a magnetic stirrer (model AA-2050, Gehaka, Brazil) at 80 °C for 30 min. After stirring, the extracts were filtered (Whatman # 1), evaporated (Rotating Evaporator TE 211, Tecnal, Brazil) and freeze-dried (FD 1.0–60, Heto, Germany). The samples remained under vacuum and under darkness prior to further use.

2.2. Burger manufacture

Four batches of lamb patties, with total replacement of animal fat by chia oil, were manufactured in the pilot plant of the Meat Technology Center of Galicia, San Cibrao das Viñas, Spain. A total of 160 burgers, weighing approximately 100 g each, were produced (4 treatments x 4 sampling points x 5 samples for each sampling point x 2 replicates). The four treatments studied were: (i) control (CONT; without antioxidant); (ii) with BHT (10 mg/kg); (iii) with guarana seed extract (G250; 250 mg/kg); (iv) with pitanga leaf extract addition (P250; 250 mg/kg). The ingredients used in all formulations were lamb meat (2 kg), salt (24 g), water (160 mL), and fat emulsion (91 g; composition: 6.7% *prosella* + 56% water + 37.3% chia oil, previously prepared by mixing the ingredients in a homogenizer for 1 min).

Prosella is a gel similar to konjac, which can be used to the total or partial replacement of animal fat by healthier oils in meat products. In its composition are included jellifying agents (E516 - E401), a stabilizer (E450, added P2O5: 9.58%), wheat glucose syrup (7.4%), and an antioxidant (E301). The elaboration of *prosella* gels were carried out the day before of the preparation of burgers. For its preparation, water (560 g/kg) and oil (372 g/kg) were mixed for one minute in a bowl cutter (Sirman, mod C15VV, Marsango, Italy) at room temperature. Then, the *prosella* powder (67 g/kg) (Prosella VG NF4, Colin Ingrédients, Mittelhausen, France) was added and homogenized during 3 min and put in a bowl. After 2 hours, the mix was jellified and was vacuum packed and kept refrigerated at 4 °C until it was used.

To produce the burgers, primal cuts of lamb leg were ground through an 8-mm diameter mincing plate (in a refrigerated mincer machine; La Minerva, Bologna, Italy), mixed, and compressed manually. Lamb patties were produced in molds of 10 cm in diameter and 1 cm in height, in a burger-maker (A-2000, Gaser, Girona, Spain) and packed in 300-mm-thick polystyrene trays, which were sealed with 74-mm-thick polyethylene film, of <2-mL/m^2 bar/day permeability (Viduca, Alicante, Spain), suitable for gas mixtures. The packaging was carried out using a heat sealer (LARI3/Pn T-VG-R-SKIN, Caveco, Palazzolo, Italy) after injection of the gas mixture containing 80% O2 and 20% CO2. The trays were stored at 2 ± 1 °C under light, to simulate supermarket conditions. Lamb burger samples were analyzed, in triplicate, at 0, 6, 12, and 18 days of storage.

2.3. Proximate composition

The procedures of the International Organization for Standards (ISO) were used to quantify the proximate composition, including protein (ISO 937, 1978), moisture (ISO 1442, 1997), and ash (ISO 936, 1998). Total fat was determined according to the Approved Procedure Am 5-04, established by the American Oil Chemists' Society (AOCS, 2005).

2.4. pH and color parameters

The pH was measured in the burgers using a digital pH-meter (Hanna Instruments, Eibar, Spain) equipped with a penetration glass probe. Color parameters were measured in the CIELAB space using a portable colorimeter (CR-600d, Minolta Co. Ltd., Osaka, Japan). The device was set to pulsed xenon arc lamp, 10° viewing angle geometry, and 8 mm aperture. The 142 metmyoglobin (MetMb) content was measured according to Krzywicki (1979) by analyzing the attenuation of the incident light at the isosbestic points at four wavelengths (572, 525, 473, and 730 nm).

2.5. Lipid and protein oxidation

Lipid stability was assessed by measuring the development of the thiobarbituric acid reactive substances (TBARs; secondary products of the lipid oxidation), and the results were expressed as milligrams of malonaldehyde (MDA) per kilogram of sample (Vyncke, 1975). Protein oxidation was determined by the carbonyl formation according to Mercier, Gatellier, and Renerre (2004). The total protein content was quantified at nm using the Biuret reagent as a standard (0 to 4 mg/mL) and the carbonyl content quantified by derivatization with dinitrophenylhydrazine (DNPH) at 370 nm. These values were calculated from a standard curve for bovine serum albumin.

2.6. *In vitro* antioxidant activity

Antioxidant activity was assayed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. One gram of lyophilized burger was homogenized with 20 mL of methanol using an IKA T25 digital Ultra-Turrax (IKA®-Werke GmbH & Co. KG, Staufen, Germany), centrifuged at 10,000 □*g* during 10 min, and filtered. The antioxidant activity values were calculated from the Trolox standard curve. A Trolox solution containing 0.03 g Trolox in 100 mL of methanol, was prepared under darkness. From this standard, seven solutions of different concentrations were obtained (from T1 = 0 mM Trolox to T7 = 1.2 mM Trolox) that were used to construct the standard curve. A 3.9 µL aliquot of 60 µM methanolic DPPH solution was added to 100 µL of the sample and Trolox solutions (T1–T7), mixed in a vortex and incubated at 37 °C for 10 min. The DPPH radical inhibition percentage was calculated by the following equation:

$$\% \text{ Inhibition} = \frac{Abs(\text{blank}) - Abs(\text{sample})}{Abs(\text{blank})} \times 100\% \quad (1)$$

where *Abs* is absorbance at 515 nm.

The absorbance value of DPPH measured at time 0 was considered as the blank (0.1 mL methanol in 3.9 mL DPPH). The antioxidant capacity of the sample was calculated by substituting the inhibition percentage in the Trolox standard curve. The results of the equivalent Trolox concentration (mM) were expressed in µg Trolox/g.

2.7. Fatty acid profile

Total fat was obtained from 10 g of sample. Total fatty acids were transesterified according to Domínguez, Crecente, Borrajo, Agregán, and Lorenzo (2015). The fatty acid methyl esters were separated and quantified using a gas chromatograph (Agilent 7890B, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector as described by Domínguez et al. (2018), and expressed as g/100 g of fat. The polyunsaturated (PUFA), monounsaturated (MUFA), and saturated (SFA) fatty acid contents; atherogenic index (AI) and thrombogenic index (TI) (Ulbricht & Southgate, 1991); and the hypocholesterolemic/hypercholesterolemic ratio (h/H) (Fernández et al., 2007) were calculated.

2.8. Analysis of volatile compounds

The volatile compounds were determined in the raw burgers at the beginning (day 1) and end of storage (day 18). For the volatile compound extraction, a solid-phase micro extraction (SPME) device (Supelco, Bellefonte, PA, USA) containing a fused silica fibre (10 mm length) coated with a 50/30mm thickness of DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) was used (Domínguez, Gómez, Fonseca, & Lorenzo, 2014a) with modifications. One gram of sample was weighed into a 20 mL vial, after being ground in a commercial grinder. The conditioning, extraction, and injection of the samples were carried out with a PAL-RTC 120 autosampler. The extractions occurred at 37 °C for 30 min following an initial temperature equilibration at the same temperature, for 15 min. After sampling, the fiber was transferred to the injection port of a gas chromatograph (7890B Agilent Technologies) equipped with a 5977B mass selective detector (Agilent Technologies) and a DB-624 capillary column (30 m x 0.25 mm i.d., film thickness 1.4 μm; J&W Scientific, Folsom, CA, USA), to determine the volatiles.

Compounds were identified by comparing their mass spectra with those contained in the NIST14 library, and/or by comparing their mass spectra and retention time with authentic standards (Supelco, Bellefonte, PA, USA); and/or by calculation of retention index relative to a series of standard alkanes (C5–C14) (for calculating linear retention index; Supelco 44585-U); and matching them with data described in the literature. The results were expressed as area units (AU) × 10^4/g of sample.

2.9. Sensory analysis

Sensory analysis was conducted by 68 consumers from Ourense (Spain). An acceptance test was realized in individual cabins under white and red lights, for the attributes evaluated in raw and cooked samples, to determine whether the panelist liked or disliked the lamb patties with total fat replacement and added natural oxidants. Panelists were asked to evaluate the following sensory attributes in either raw patties (color, discoloration at surface, and odor) or cooked (odor and taste) using a 5-point hedonic scale (1 = excellent; 5 = not acceptable), according to Lago et al. (2017), but

in reverse order. In addition, the panelists were asked to order the samples according to their preference. The sensory sessions to assess the raw patties were carried out at 0, 6, 12, and 18 days of storage. The patties were cooked in an oven (Rational CombiMaster®Plus CMP61, Landsberg am Lech, Germany) equipped with a core temperature probe, until they reached an internal temperature 217 of 70 °C. The samples were presented to each panelist in plastic dishes coded with 3-digit random numbers (MacFie, Bratchell, Greenhoff, & Vallis, 1989). Water and unsalted toasted bread were used at the beginning of sessions and between samples to clean the palate and remove residual flavors.

2.10. Statistical analysis

A completely randomized design was used with four treatments (CONT, BHT, G250, P250; see section 2.1) and five repetitions. A total of 40 burgers were analyzed in each point: five burger samples for each batch x four batches (CONT, BHT, G250, and P250) x two replicates, using the mathematical model:

$$Y_{ijk} = \mu + S_i + T_j + ST_{ij} + \mathrm{e}ijk \quad (2)$$

where *Yijk* is the *k*-th observation of the dependent variables (*k* = 1, 2, 3) for the *i*th storage time (*i* = 1, 2, 3, 4) and *j*-th treatment (*j* =1, 2, 3, 4); *μ* is the overall mean; *Si* is the effect of storage time; *Tj* is the effect of the treatments; *STij* is the interaction between treatments and storage time; and eijk is the random error associated with the *Yijk* observation. Normal distribution and variance homogeneity were previously tested (Shapiro–Wilk). The effect of treatments and time were examined using a two-way ANOVA. Chemical composition was only analyzed at day 0 using a one-way ANOVA. When a significant effect ($P < 0.05$) was detected, means were compared using Tukey's test. Statistical analyzes were carried out using SAS (version 9.4, SAS Institute Inc., Cary, NC, USA).

3. RESULTS AND DISCUSSION

3.1. Proximate composition

As expected, the chemical composition did not significantly differ among treatments ($P > 0.05$), showing mean values of (72.52-72.82%) for moisture, (6.47-661%) for fat, (17.23-17.48%) for protein, and (2.34-2.35%) for ash (data not shown). These results agree with data reported by other authors (Carvalho et al., 2019; Marti-Quijal et al., 2019; Zugcic et al., 2018) in burger meat.

3.2. pH and color parameters

The effect of guarana seed and pitanga leaf extracts on pH and color parameters of lamb patties during cold storage is shown in Table 1. The results showed an initial decrease in pH values ($P < 0.01$), but after 6 days, the pH remained constant until the end of storage, which could be due to degradation of proteins and liberation of peptides, amino acids, ammonia and amines during the shelf life. Among batches, higher pH values (pH 5.80 and 5.82 on day 0) were found in G250 and P250 treatments, respectively. These findings are consistent with previous data reported by Lorenzo, Sineiro, Amado, and Franco (2014) and Lorenzo et al. (2018), who observed a reduction in the pH values in burgers elaborated with grape and pitanga extracts, respectively.

Table 1. Effect of guarana seed and pitanga leaf extracts on pH and color parameters of lamb patties with replacement of animal fat by chia oil during refrigerated storage.

		Days of storage					
	Treatment	0	6	12	18	SE	Sig.
pH	Control	5.72bA	5.54bB	5.51bB	5.54aB	0.05	***
	BHT	5.75bA	5.55bB	5.53abB	5.58aB	0.04	***
	G250	5.80aA	5.62aB	5.56abB	5.55aB	0.05	***
	P250	5.82aA	5.62aB	5.59aB	5.58aB	0.08	***
	SE	0.01	0.02	0.19	0.07	-	-
	Sig.	***	***	*	ns	-	-
L*	Control	53.07aAB	51.61aB	55.26aA	55.22aA	0.78	**
	BHT	52.870aBC	51.13aC	55.72aA	54.56abAB	0.83	**
	G250	52.00aA	48.50bB	51.14bA	53.01bcA	0.92	***
	P250	50.51aAB	48.56bB	50.36bAB	52.20cA	0.76	*
	SE	1.09	0.50	0.34	0.38	-	-
	Sig.	ns	*	***	**	-	-
a*	Control	11.04bA	5.94cB	4.05bC	3.51bC	0.25	***
	BHT	10.96bA	7.13bB	3.96bC	4.03bC	0.32	***
	G250	12.74aA	8.36aB	5.60aC	4.83aC	0.39	***
	P250	11.76abA	7.62abB	5.80aC	4.89aC	0.23	***
	SE	0.56	0.37	0.31	0.29	-	-
	Sig.	*	***	***	***	-	-
b*	Control	16.66bA	14.95aB	17.09aA	16.84aA	0.39	***
	BHT	16.20bB	15.21aC	17.33aA	16.82aAB	0.69	***
	G250	17.93aA	14.80aC	15.45bC	16.43aB	0.52	***
	P250	16.77bA	13.56bC	15.06bB	15.96aA	0.78	***
	SE	0.44	0.41	0.33	0.89	-	-
	Sig.	**	**	***	ns	-	-
MetMb	Control	21.65bC	49.15aB	62.11aA	62.28aA	0.27	***
(%)	BHT	21.37bC	45.25bB	62.21aA	62.65aA	0.59	***
	G250	24.45aD	38.93cC	56.98bB	61.98aA	0.94	***
	P250	24.54aD	39.08cC	56.77bB	61.87aA	1.71	***
	SE	0.70	1.17	1.28	0.73	-	-

Sig.	***	***	***	ns	-	-

A-D Mean values in the same row (same antioxidant in different days) with different letters indicate significant difference; a-c Mean values in the same column (different antioxidant in same day) with different letters indicate significant difference. SE: Standard error; Sig.: significance; n.s.: not significant; ∗ P<.05. ∗∗ P<.01 ∗∗∗ P<.001;

The color parameters were significantly affected by the treatments and storage time. In general, L* values increased during the whole display, reaching final values higher than at the beginning. Our results agree with data reported by Lorenzo et al. (2018) who observed increases in L* values during storage of pork patties incorporated with guarana seed extract. On the other hand, all treatments presented a significant decrease in a* values as storage progressed, showing the highest a* values ($P < 0.05$) in burgers manufactured with natural antioxidants. At the end of storage, G250 and P250 presented more red intensity than the CONT and BHT treatments (4.83 and 4.89 *vs*. 3.51 and 4.03, respectively). These results can probably be explained by lower oxidation of the heme group within the iron atom and a lower formation of MetMb (Faustman, Sun, Mancini, & Suman, 2010), confirming the findings obtained for MetMb (Table 1). In this regard, Fernandes, Trindade, Lorenzo, Munekata, and de Melo (2016b) also noticed a similar result for lamb burger added with oregano extract.

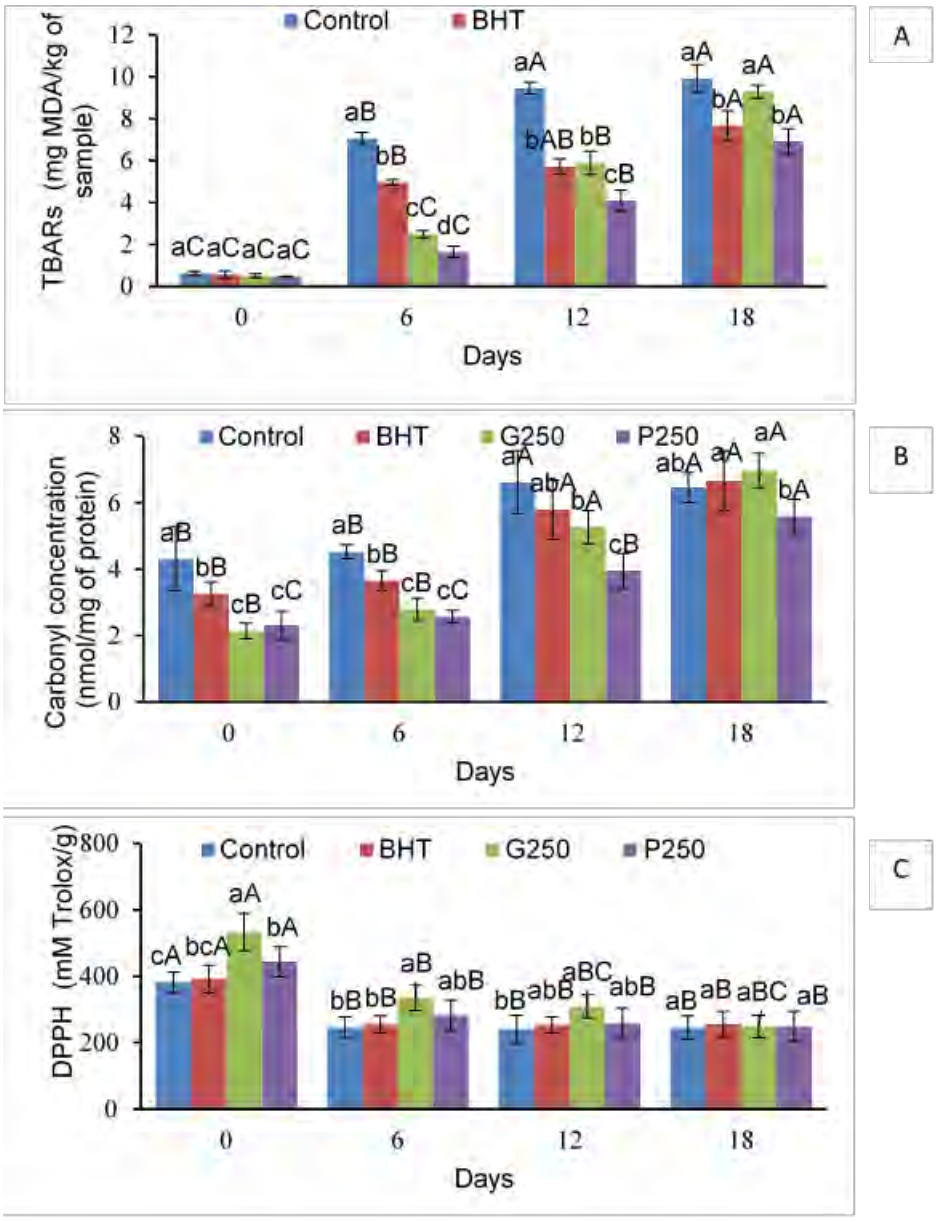
Statistical analysis displayed significant increases in MetMb contents during storage, with values ranging from 21.37% to 24.54% on day 0, and from 61.87% to 62.65% on day 18. Greene, Hsin, and Zipser (1971) claimed that at above 40%, MetMb could influence consumers' purchasing intention. The CONT and BHT treatments had already reached these values at day 6 of storage (49.15% and 45.24%, respectively), whereas, G250 and P250 treatments delayed this increase until day 12 (56.98% and 56.77%, respectively). This finding agrees with data reported by Pateiro et al. (2018) who noticed that guarana seed extract delayed MetMb formation compared to control and BHT in pork patty samples.

3.3. Lipid and protein oxidation of lamb burgers

The changes in the TBARs content of lamb burgers throughout storage time (Fig. 1A), indicated that the TBARs levels were significantly ($P < 0.05$) affected by the

storage time and antioxidant addition. As could be expected, the MDA content increased as storage progressed, regardless of the type of treatment. The CONT had TBARs values ranging from 0.61 to 9.92 mg MDA/kg, whereas in the other treatments, the range was narrower. The extract of pitanga was more efficient in retarding the lipid oxidation than the other treatments in all sampling points studied. Until day 12, the lipid oxidation was significantly ($P < 0.01$) lower in G250 group than BHT treatment. At the end of storage, P250 and BHT treatments presented the least lipid oxidation ($P < 0.01$).

Figure 1. Evolution of TBARs values (A), total carbonyl content (B) and antioxidante activity (C) in lamb patties elaborated with BHT, guarana seed, and pitanga leaf extracts during storage. Error bars corresponding to standard error. Different lowercase letters indicate a significant difference between treatments and different capital letters indicate a significant difference in different days.



Lipid oxidation is the main process responsible for the quality deterioration of meat and meat products by reducing shelf life. Oxidation increased rapidly, such that on day 6, the CONT, BHT, and G250 treatments had already exceeded the limit of acceptability (2 mg MDA/kg) posed by Greene and Cumuze (1982), whereas the addition of pitanga extract retarded the lipid oxidation. The antioxidant properties of

guarana and pitanga extracts are related to their polyphenols content. Lorenzo et al. (2018) and Pateiro et al. (2018) noticed vast quantities of total polyphenols in guarana (28.2 g/kg) and pitanga (35.13 g/kg) extracts, respectively. Besides the quantity, the type of phenolic compound and its mechanism of action are key factors to explaining the antioxidant activity of an extract, which, Rice-Evans, Miller, and Paganga (1996) affirm, is determined by the number and positions of the hydroxyl groups in association with the functional carboxyl. This phenomenon could explain the comparatively greater inhibition presented by the pitanga extract, among the studied treatments.

The evolution of total carbonyl content of lamb burgers during display (Fig. 1B) evidenced a significant ($P < 0.05$) increase in the protein carbonyl during storage, regardless of treatment, with results varying from 2.14 to 4.30 nmol/mg at day 0, and from 5.59 to 6.97 nmol/mg at 18 days. Burgers with antioxidants showed lower carbonyl contents when compared with CONT treatment. The P250 group presented the lowest carbonyl contents during the whole display, similar to the trend observed in the TBARs values. Only at the end of storage, G250 and BHT treatments displayed comparable protein oxidation to CONT group. These facts evidence the effectiveness of antioxidants, especially natural antioxidants, and corroborate the work of other authors (Fernandes et al., 2017; Lorenzo et al., 2018; Pateiro et al., 2018) who noticed that natural extracts presented more antioxidant activity than synthetic antioxidants, suggesting the possibility of using these extracts as replacers of commercial additives.

According to Pateiro et al. (2018), guarana seed extract contains high amounts of phenolic compounds responsible for protective effects against oxidation process. In addition, Lorenzo et al. (2018) also detected a high total phenolic content of 229.38 mg gallic acid equivalents/g in pitanga left extracts. However, the antioxidant activity of phenolic compounds depends on the number and position of the hydroxyl groups linked to the aromatic ring, and are capable of donating hydrogen atoms or acting as eléctron neutralizing free radicals (Krishnann et al., 2014).

3.4. *In vitro* antioxidant activity

The DPPH antioxidant activities of lamb burger decreased significantly ($P < 0.05$) during cold storage (Fig. 1C). The natural antioxidants (P250 and G250 treatments) maintaining a greater antioxidant activity compared to CONT and BHT batches until days 6 and 12, respectively. At 18 day, all groups presented similar antioxidant capacity to each other ($P > 0.05$). Mancini et al. (2015), who tested the addition of turmeric powder in rabbit burger, also noticed a reduction in the antioxidant power over time (from 0 to 7 days) correlating with an increased extent of lipid oxidation, not dissimilar to the behavior found in this study.

3.5. Fatty acid profile

Examination of the fatty acid profile in lamb burger at the beginning and end of storage (Table 2) revealed, unsurprisingly, no difference ($P > 0.05$) among treatments for any studied fatty acid at day 0. This fact could be due to the emulsion of chia oil prevents its oxidation. However, there was variation among fatty acids over time, and differences among treatments at the end of storage time. In all treatments the major fatty acids were the MUFA (means of 40.95% and 42.24%) followed by the SFA (means of 36.92% and 37.08%) and the PUFA (means of 20.88% and 19.36%), on days 0 and 18, respectively. With the replacement of animal fat by emulsified chia oil, there was an improvement in the fatty acid profile. This result agrees with data reported by Heck et al. (2017); and Pintado, Ruiz-Capillas, Jiménez-Colmenero, Carmona, and Herrero (2015) who noticed a decreased in SFA and MUFA levels and an increased in PUFA content in meat products with port back fat replaced by 342 chia oil. However, the burgers could be more susceptible to oxidation since susceptibility to oxidation increases with an increasing number of double bonds in fatty acids, and so the impact of antioxidants would be evident.

Individually, palmitic acid (C16:0) was the most abundant SFA; G250 and P250 exhibited the highest values (19.93% and 20.30%, respectively) at day 18 while oleic acid (18:1n-9*cis*) was the predominant MUFA, and increased from day 0 to day 18 when P250 displayed the highest value (31.51%). These results agree with those

published by Heck et al. (2017), who obtained the same major fatty acids in beef Burger with chia oil microparticles.

Table 2. Fatty acids profile in sheep burgers prepared with BHT, guarana seed (G250), and pitanga leaf (P250) extracts during storage (0 and 18 days) at 2 °C.

Fatty acid	Day	Treatment				SE	Sig.
		Control	BHT	G250	P250		
C14:0	0	2.55	2.51	2.57	2.56	0.03	ns
	18	2.62	2.63	2.65	2.68	0.05	ns
	Sig.	ns	*	ns	**	-	-
C15:0	0	0.66	0.64	0.64	0.64	0.01	ns
	18	0.66	0.66	0.66	0.67	0.01	ns
	Sig.	ns	ns	*	*	-	-
C16:0	0	19.46	19.09	19.30	19.22	0.14	ns
	18	19.68ab	19.45b	19.93ab	20.30a	0.23	**
	Sig.	ns	ns	***	***	-	-
C16:1n-7	0	1.37	1.35	1.38	1.35	0.02	ns
	18	1.39b	1.39b	1.41ab	1.45a	0.02	**
	Sig.	ns	*	ns	***	-	-
C17:0	0	2.35	2.28	2.28	2.27	0.04	ns
	18	2.38	2.36	2.35	2.40	0.04	ns
	Sig.	ns	ns	*	**	-	-
C17:1n-7	0	1.28	1.24	1.24	1.22	0.03	ns
	18	1.28	1.28	1.26	1.32	0.03	ns
	Sig.	ns	ns	ns	**	-	-
C18:0	0	12.16	11.95	12.09	12.14	0.13	ns
	18	11.95	12.36	11.90	10.63	0.70	ns
	Sig.	ns	**	ns	*	-	-
11t-C18:1	0	7.04	6.89	6.90	7.02	0.14	ns
	18	7.09	7.08	7.09	7.11	0.11	ns
	Sig.	ns	ns	*	ns	-	-
C18:1n-9	0	30.19	30.00a	29.90	29.86	0.27	ns
	18	30.87ab	30.36b	30.62ab	31.51a	0.36	*

	Sig.	*	*	*	***	-	-
C18:1n-7	0	1.22	1.38	1.36	1.39	0.01	ns
	18	1.45	1.39	1.45	1.46	0.03	ns
	Sig.	ns	ns	*	*	-	-
C18:2n-6	0	7.92	8.11	7.99	8.02	0.13	ns
	18	7.52	7.58	7.61	7.58	0.14	ns
	Sig.	*	**	**	*	-	-
C20:1n-9	0	0.17	0.17	0.17	0.17	0.02	ns
	18	0.17ab	0.17b	0.18ab	0.18a	0.03	*
	Sig.	ns	ns	*	**	-	-
C18:3n-3	0	10.96	11.81	11.49	11.49	0.40	ns
	18	10.81	11.14	10.52	10.28	0.37	ns
	Sig.	ns	ns	**	**	-	-
9c,11t-C18:2	0	0.35	0.35	0.35	0.36	0.01	ns
	18	0.36	0.36	0.35	0.36	0.01	ns
	Sig.	ns	ns	ns	ns	-	-
C20:4n-6	0	0.90	0.91	0.86	0.87	0.03	ns
	18	0.52b	0.53b	0.67a	0.69a	0.02	***
	Sig.	**	**	***	**	-	-
C22:5n-3	0	0.19	0.19	0.19	0.19	0.01	ns
	18	0.13b	0.13b	0.15a	0.16a	0.01	***
	Sig.	***	***	***	**	-	-
C22:6n-3	0	0.10	0.10	0.09	0.09	0.01	ns
	18	0.04b	0.04b	0.05a	0.06a	<0.01	***
	Sig.	***	***	***	***	-	-
SFA	0	37.18	36.47	37.18	36.83	0.30	ns
	18	37.31	37.46	36.88	36.67	0.53	ns
	Sig.	ns	*	ns	ns	-	-
MUFA	0	41.28	40.57	40.95	41.01	0.43	ns
	18	42.25	41.66	42.01	43.04	0.47	ns
	Sig.	ns	*	*	***	-	-
PUFA	0	20.31	21.37	20.92	20.93	0.55	ns
	18	19.32	19.75	19.30	19.08	0.50	ns

	Sig.	ns	*	**	**	-	-
PUFA/SFA	0	0.55	0.59	0.57	0.57	0.02	ns
	18	0.52	0.53	0.52	0.52	0.02	ns
	Sig.	ns	*	**	ns	-	-
n-3	0	11.24	12.10	11.78	11.77	0.40	ns
	18	10.97	11.31	10.72	10.50	0.38	ns
	Sig.	ns	ns	**	**	-	-
n-6	0	8.81	9.02	8.88	8.89	0.16	ns
	18	8.03	8.12	8.28	8.25	0.15	ns
	Sig.	**	***	**	*	-	-
n-6/n-3	0	0.79	0.75	0.75	0.76	0.01	ns
	18	0.74bc	0.72c	0.78ab	0,79a	0.02	***
	Sig.	*	*	ns	ns	-	-
AI	0	0.48	0.46	0.48	0.48	0.01	ns
	18	0.49	0.49	0.50	0.50	0.01	ns
	Sig.	ns	ns	**	***	-	-
TI	0	0.57	0.54	0.55	0.55	0.01	ns
	18	0.58	0.57	0.59	0.57	0.02	ns
	Sig.	ns	ns	*	ns	-	-
h/H	0	2.27	2.34	2.30	2.31	0.03	ns
	18	2.23	2.25	2.19	2.18	0.03	ns
	Sig.	ns	ns	***	***	-	-

a-c Mean values in the same row with different letters indicate significant difference; SE: Standard error; Sig.: significance; n.s.: not significant; ∗ P<.05. ∗∗ P<.01 ∗∗∗ P<.001;

Regarding nutritional aspects, the PUFA/SFA ratio decreased over time in BHT and P250 groups but did not differ (P >0.05) from the other treatments, with a mean value of 0.57 and 0.52 at days 0 and 18, respectively. These levels were below that established by the Food and Agriculture Organization of the United Nations (FAO, 2010) for human nutrition (0.85). The n-3 content in G250 and P250 treatments decreased significantly (P < 0.01) from day 0 to day 18, due to the decrease in C18:3n-3 content. Consequently, there was a change in the n-6/n-3 ratio of these treatments, which presented the highest values (0.78 and 0.79; P < 0.01, for G250 and P250 treatments, respectively). Even so, all treatments were below the maximum limit of

4.00 recommended by the FAO (2010) without significant differences ($P > 0.05$) among treatments.

The incorporation of natural extracts changed the AI, TI, and h/H ratio over time. At the end of storage, G250 group presented an increased in TI (0.50) and AI (0.59) values and a decreased in h/H ratio (2.19) compared with day 0 (0.48, 0.55, and 2.30, for AI, TI, and h/H ratio, respectively). This tendency was different to that observed for the P250 treatment, since the AI dropped 367 from 0.50 (day 0) to 0.48 (day 18) while the h/H ratio increased from 2.18 (day 0) to 2.30 (day 18). The AI and TI are used to express the effects of each fatty acid on human health. Very low levels of these indices are recommended for a healthy diet (Ulbricht & Southgate, 1991). Our h/H ratios were higher than those observed in lamb (Quiñones et al., 2019), evidencing the improvement in the fatty acid profile obtained with the substitution of fat by chia oil emulsion.

At first glance, the efficacy of the natural extracts seemed to be compromised over time, but when compared with CONT and BHT treatments, there was no differences ($P > 0.05$) in AI, TI, and h/H at the beginning or at the end of storage, indicating that the use of natural extracts did not modify the results from the nutritional point of burgers. In addition, Heck et al. (2017) reported similar AI (0.45) and TI (0.58) values in beef hamburger with fat replacement by chia oil microparticles.

3.6. Analysis of volatile compounds

Selected volatile compounds from lamb burgers, evaluated at the start and the end of storage, are shown in Table 3. The addition of oils with an increased amount of unsaturated fatty acids favors oxidative processes of meat and, consequently, increases the volatile compounds arising from lipid oxidation (Kumar, Yadav, Ahmad, & Narsaiah, 2015).

The major chemical families of volatile compounds, as products of oxidation, were alcohols, ketones, and aldehydes, concurring with the volatile profile of lamb and lamb burger (Fernandes et al., 2016b). These chemical families were primarily responsible for explaining the oxidation during cooking lamb burger in a principal

component analysis of the unsaturated fatty acids and volatile compounds derived from lipid oxidation (Bravo-Lamas, Barron, Farmer, & Aldai, 2018). Aldehydes and ketones are well-recognized as the main 391 aroma compounds resulting from lipid oxidation (Resconi, Escudero, & Campo, 2013).

Table 3. Volatile compounds in sheep burgers prepared with BHT, guarana seed (G250), and pitanga leaf (P250) extracts during storage (0 and 18 days) at 2 °C expressed as area units (AU) × 10^4/g of $sample^{-1}$.

	m/z	LRI	R	Day	Treatments				SE	Sig.
					Control	BHT	G250	P250		
Aldehydes										
2-Octenal, (E)-	112	833	ms, lri	0	0.80	0.38	0.59	0.67	0.18	ns
				18	2.91a	0.89b	0.51b	0.41b	0.42	***
				Sig.	***	**	ns	ns		
Heptanal	70	974	ms, lri, s	0	1.07	1.00	1.15	1.10	0.31	ns
				18	110.19a	15.90b	14.93b	3.69b	2.11	***
				Sig.	***	ns	ns	***		
2-Heptenal, (E)-	83	1068	ms, lri	0	3.94	2.76	3.70	3.64	0.59	ns
				18	9.02a	4.91b	5.34b	4.67b	1.01	***
				Sig.	***	*	ns	ns		
2-Nonenal, (E)-	55	1247	ms, lri	0	1.45a	0.6.47b	1.5.25a	1.50a	0.23	**
				18	6.32a	2.45b	1.67bc	0.59c	0.64	***
				Sig.	***	***	ns	***		
Octanal	56	1066	ms, lri, s	0	3.09	2.82	3.55	2.68	0.75	ns
				18	9.61	5.45	6.16	3.24	2.44	ns
				Sig.	***	*	ns	ns		
Nonanal	57	1148	ms, lri	0	3.85a	1.26b	2.63ab	2.58ab	0.82	*

				18	16.19a	4.08b	1.83b	1.37b	1.20	***
				Sig.	***	***	ns	**		
Pentanal	57	728	ms, lri, s	0	23.58a	4.16b	4.43b	6.29b	1.82	**
				18	43.18a	33.54ab	15.05bc	5.83c	7.12	***
				Sig.	*	**	*	ns		
Hexanal	56	865	ms, lri	0	15.49a	3.18b	14.48a	13.54a	3.66	**
				18	112.75a	18.92b	9.76b	4.79b	18.78	***
				Sig.	***	***	ns	**		
Ketones										
2-Heptanone	58	967	ms, lri	0	22.46a	20.51a	18.66a	15.33b	1.96	**
				18	57.94	53.14	58.47	56.11	3.44	ns
				Sig.	***	***	**	**		
2-Pentanone	86	720	ms, lri	0	11.61	11.85	13.13	11.3.45	1.58	ns
				18	17.76b	17.15b	22.03ab	27.62a	3.01	**
				Sig.	*	**	*	**		
2-Octanone	58	1059	ms, lri	0	13.18	8.90	14.41	11.84	2.08	ns
				18	50.34	45.75	46.10	50.99	2.96	ns
				Sig.	***	***	**	**		
Alcohols										
1-Hexanol	56	969	ms, lri	0	143.59a	90.36b	133.48ab	79.65b	19.70	**
				18	737.35a	540.10b	504.23b	379.63c	28.70	***

				Sig.	***	***	***	**		
1-Octanol	56	1127	ms, lri	0	4.51bc	1.79c	5.22b	5.61a	1.07	**
				18	24.07a	8.77b	5.07bc	2.20c	1.49	***
				Sig.	***	***	ns	*		
1-Butanol	56	707	ms, lri	0	1.21b	1.05b	2.11a	1.47b	0.56	***
				18	4.55	3.71	4.18	3.89	2.45	ns
				Sig.	***	***	*	***		
1-Pentanol	55	847	ms, lri	0	55.93a	36.65c	53.98ab	39.42bc	5.85	**
				18	245.98a	163.19b	142.72b	105.38c	11.51	***
				Sig.	***	***	***	***		
1-Octen-3-ol	57	1051	ms, lri	0	197.94a	144.80b	186.07ab	150.87ab	18.95	*
				18	551.50a	384.38b	391.28b	326.23b	30.45	***
				Sig.	***	***	***	**		
1-Penten-3-ol	57	730	ms	0	75.71ab	39.66b	148.42a	156.48a	35.44	**
				18	190.57	135.62	168.22	116.03	37.12	ns
				Sig.	***	***	ns	ns		

a-c Mean values in the same row with different letters indicate significant difference; SE: Standard error; Sig.: significance; n.s.: not significant; * P<0.05; ** P<0.01; ** P<0.001 m/z: Quantification ion; LRI: Lineal Retention Index calculated for DB-624 capillary column (J&W scientific: 30m×0.25mm id, 1.4 µm film thickness) installed on a gas chromatograph equipped with a mass selective detector; R: Reliability of identification; *lri*: linear retention index in agreement with literature (Dominguez et al., 2014a; Lorenzo, 2014; Lorenzo, Bedia, & Banon, 2013; Lorenzo & Carballo, 2015; Lorenzo & Dominguez, 2014; Lorenzo, Montes, Purrinos, & Franco, 2012; Pateiro, Franco, Carril, & Lorenzo, 2015; Perez-Santaescolastica et al., 2018a; Perez-Santaescolastica et al., 2018b; Purrinos et al., 2011; Purrinos, Carballo, & Lorenzo, 2013; Purrinos, Franco, Bermudez, Carballo, & Lorenzo, 2011; Purrinos, Franco, Carballo, & Lorenzo, 2012); *ms*: mass spectrum agreed with mass database (NIST14); *s*: mass spectrum and retention time identical with an authentic standard.

In the present study, CONT group exhibited a significant increase in the levels of all aldehydes studied, whereas the increase was not so dramatic in BHT treatment. The G250 group did not show changes in the concentrations of the analyzed aldehydes, except for the pentanal content, which was higher at the end of storage (15.05 AU x 10^4/g) than at day 0 (4.43 AU x 10^4/g), but lower than the amounts detected in CONT treatment (23.58 AU x 10^4/g at day 0 vs. 43.18 AU x 10^4/g at day 18). In P250 group, (*E*)-2-heptenal, octanal, and pentanal levels were not affected ($P > 0.05$), however, heptanal, (*E*)-2-nonenal, and hexanal contents were reduced over time. These compounds are derived from lipid oxidation, with the production of aldehydes from phospholipids and PUFAs. Some of these aldehydes have unpleasant odors even in very low concentrations. Pentanal, for example, is negatively correlated with taste quality and may indicate taste impairment (Stetzer, Cadwallader, Singh, Mckeith, & Brewer, 2008). At the end of storage, the pentanal content was lower in P250 treatment than in CONT and BHT groups.

Hexanal is considered one of the main indicators of lipid oxidation in meat and meat products. It imparts a rancid taste when found in high concentrations (Brunton, Cronin, Monahan, & Durcan, 2000) and is the most prevalent among the aldehyde family (Maggiolino et al., 2019). During the storage time, the hexanal increased in the CONT and BHT groups, and decreased in P250 and G250 treatments. As a result, at day 18, treatments with antioxidants (synthetic and natural) had less hexanal compared with CONT group, demonstrating that the guarana and pitanga extracts had the same potential to prevent lipid oxidation as the synthetic antioxidant.

In general, the content of the alcohols (1-hexanol, 1-octanol, 1-butanol, 1-pentanol, 1-octen-3-ol, 1-penten-3-ol) in the burgers increased during refrigerated period. These results substantiate the literature data that demonstrated increases in 1-hexanol and 1-octen-3-ol during storage (Watanabe et al., 2015), which, as the TBARs values indicated in the present study, is accompanied by increasing lipid oxidation. Within the alcohol group, 1-hexanol, 1-octen-3-ol, and 1-pentanol are the volatile compounds most frequently reported in the literature and considered as the key indicators of lipid oxidation in meat (Maggiolino et al., 2018; Resconi et al., 2018). The addition of antioxidants, regardless of the source, was able to reduce the content of these compounds in comparison to CONT group. In addition, at the end of the storage,

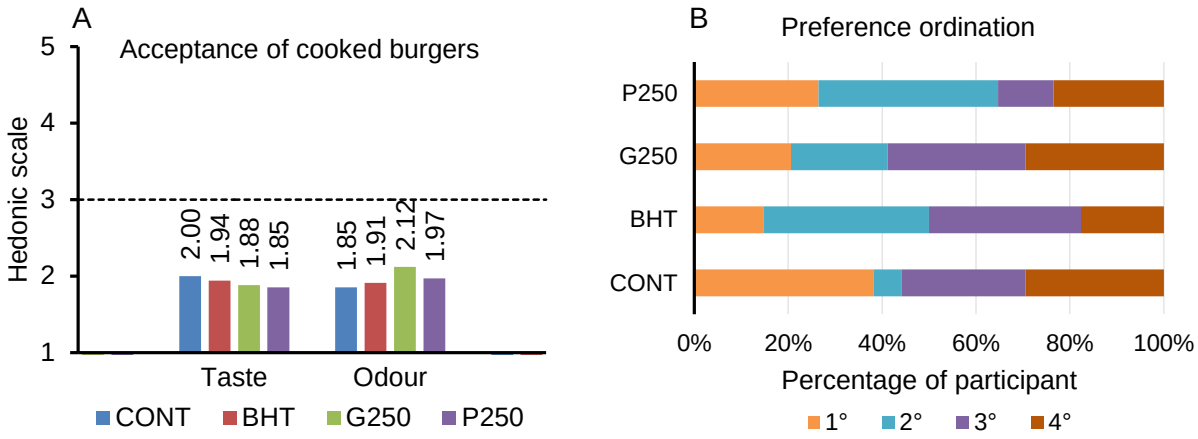
the P250 treatment was more efficient in avoiding the formation of 1-hexanol, 1-octen-3-ol, and 1-pentanol, presenting the lowest values among all the treatments studied. On the contrary, Fernandes et al. (2016b) observed no effect of oregano extract on the content of 1-pentanol over time in relation to BHT and CONT groups and did not find 1-hexanol and 1-octen-3-ol in sheep burger.

Certain ketones have been correlated with intramuscular fat content (Machiels, Istasse, & van Ruth, 2004) and flavor modifications in lamb, with some of them providing lamb with meaty flavors (Carballo, Caro, Andrés, Giráldez, & Mateo, 2018). However, a positive correlation between 2-heptanone and 2-octanone with TBARs index was reported (Domínguez, Gómez, Fonseca, & Lorenzo 2014b; Resconi et al., 2018). In the present study, the amount of ketones (2-heptanone, 2-pentanone, and 2-octanone) increased over time in all treatments ($P < 0.05$), although there was no difference between them on day 18. According to Bravo-Lamas et al. (2018), 2-heptanone and 2-pentanone are among the main compounds responsible for the variation in cooked lamb oxidation and are 439 oxidation compounds of linoleic and α-linolenic acids, respectively.

3.7. Sensory analysis

From the sensory analysis performed on the cooked product (Fig. 2A), there was no difference ($P > 0.05$) in the flavor or aroma of the burgers, and all treatments received an acceptance test score lower than 3 (acceptability limit), indicating that the addition of guarana and pitanga extracts did not alter the acceptability of cooked lamb burger. Regarding preference (Fig. 2B), most of the panelists chose CONT group (38.3%), followed by P250 (26.5%), G250 (20.6%) treatments, whereas the least preferred was BHT batch (14.7%). When considering the percentage of tasters who chose each treatment as the first and second option (sum of the two), the order was P250 (64.7%), followed by BHT (50%), CONT (44.1%), and G250 (41.2%). In both cases, P250 treatment appears as one of the most preferred among tasters, indicating that this extract can be used without impairing the sensory characteristics of lamb burgers.

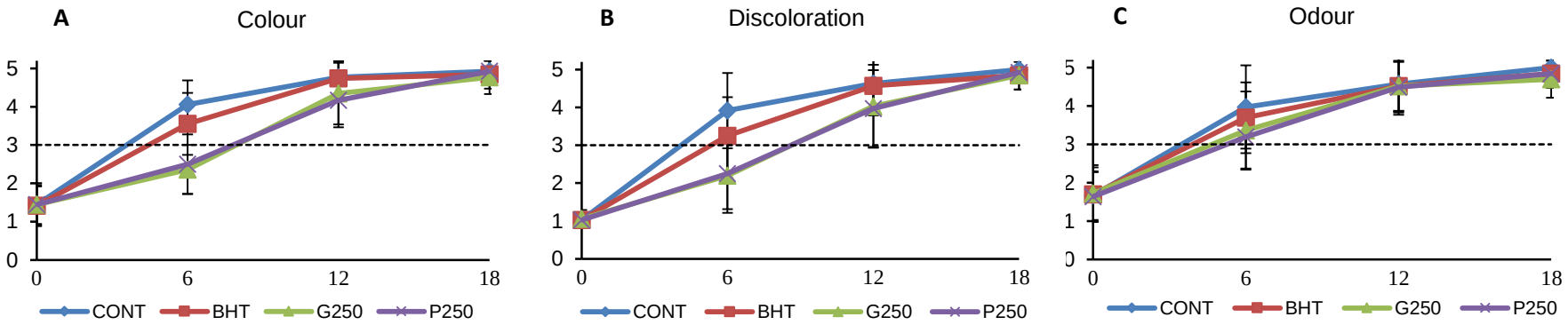
Figure 2. Sensory scores (**A**) and preference (**B**) attributed by the panelist on day 0 for cooked lamb patties produced with BHT, guarana seed, and pitanga leaf extracts. Hedonic scale used: 1 = excellent; 2 = good; 3 = acceptable; 4 = hardly acceptable; 5 = not acceptable. Error bars corresponding to standard error.



For the sensorial analysis undertaken on the raw product during its shelf life, guarana and pitanga extracts maintained the color of the burgers at acceptable levels until day 6, whereas the color of CONT and BHT treatments was already unacceptable after day 1 (Fig. 3A). In addition, natural antioxidants were able to delay the perception of discoloration by the panelists until day 6 as a consequence of the prevention of myoglobin oxidation (Fig. 3B). Subsequently, both color and discoloration presented unacceptable levels in all treatments at the end of storage period. This fact was reflected by the redness values measured in the respective treatments, as P250 and G250 treatments had higher a* values throughout storage time and only decreased significantly at day 6 when the testers detected this difference. This result is importante since the decision to purchase meat and products is greatly 463 influenced by color because consumers use discoloration as an indicator of freshness and integrity.

On day 6, the odor of burgers already received scores between 3 (acceptable) and 5 (not acceptable). However, this behavior was not affected by the treatment or the storage period. Thus, there was no modification in this attribute by the addition of the natural extracts. Heck et al. (2017) reported sensory impairment with the replacement of fat by chia oil in beef burger, probably due to the high PUFA level of the chia oil, thereby deeming the burger more susceptible to lipid oxidation.

Figure 3. Sensory scores attributed by the panelist on days 0, 6, 12, and 18 for (**A**) color, (**B**) discoloration, and (**C**) odor of raw lamb burgers produced with BHT, guarana seed, and pitanga leaf extracts during storage. Hedonic scale used: 1 = excellent; 2 = good; 3 = acceptable; 4 = hardly acceptable; 5 = not acceptable. Error bars corresponding to standard error.



4. CONCLUSION

Guarana seed and pitanga leaf extracts may be substitutes for synthetic antioxidant in lamb burgers, without causing detrimental changes in their physical chemical and sensory properties. The extracts suppressed the loss of redness by delaying discoloration, which can influence the purchase decision. Moreover, both extracts displayed greater antioxidant activity in protecting the burgers against lipid and protein oxidation during storage time than BHT, as confirmed by the decreased of volatile compounds from lipid oxidation, especially with pitanga extract. The fatty acid profile of lamb burgers was improved by fat replacement by chia oil emulsion. Thus, this study demonstrated that guarana seed and pitanga leaf extracts are promising natural antioxidants for increasing the shelf life of meat products, even in burgers most susceptible to oxidation.

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CHAPTER II - Effect of replacing backfat with vegetable oils during the shelf-life of cooked lamb sausages*

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Effect of replacing backfat with vegetable oils during the shelf-life of cooked lamb sausages

Abstract

The effects of chia, linseed, and olive oils as backfat replacers on the physico-chemical, oxidative stability and sensory attributes of lamb sausages storage at 2 °C were assessed. Four batches were studied: a control (CONT; with pork fat); and three with fat replaced by commercial vegetable oils: chia (CHIA), linseed (LINS), and olive (OLIV). The incorporation of vegetable oils led to consistent improvement in the fatty acid profile and nutritional indexes. CHIA, LINS, and OLIV batches decreased the atherogenicity (IA) and thrombogenicity (IT) indexes ($P<0.05$); however, only the CHIA and LINS groups showed a n-6/n-3 ratio (0.86 and 0.92, respectively) and PUFA/SFA within the recommended. Regarding sensory analysis of the cooked products, LINS treatment did not differ from CONT group, while CHIA and OLIV batches caused damage to taste. During storage, there was no difference in the color, discoloration, and odor of the raw products ($P>0.05$). The CONT group presented the highest a* value over time, but this was not noticed by the panelists. CHIA and OLIV batches had an increase in the hardness over time. Protein oxidation increased until day 60; showing only significant difference at the beginning of the display since OLIV group presented the highest value (7.96 nmol carbonyl/mg).

Keywords: chia, linseed, olive, fat replacement, volatile compounds.

1. **INTRODUCTION**

Cooked sausages are traditional meat products, mainly elaborate with pork lean and fat, salt, garlic, sugar, nitrite, and different spices (Ayo, Carballo, Solas, & Jimenez-Colmenero, 2008). Generally, the fat content in sausages can range up to 30% animal fat, which in turn has a great influence on the formation of stable emulsions, cooking loss, retention capacity of water, providing rheological and structural properties responsible for the succulence, texture, and physicochemical properties of the products (Saldaña et al., 2015). However, fat and/or cholesterol content are major concerns in processed meat products (Monteiro, Souza, Costa, Faria, & Vicente, 2017), and many consumers are aware of the health risks due to high intake of animal fat; so, the demand of low-fat meat products is growing (da Silva et al., 2019). The increasing concerns for healthier meat products have given rise to the reformulation of this type of products (Sevi, Marino, Lorenzo, Picard, & Pereira, 2016; Bis-Souza, Barba, Lorenzo, Penna, & Barretto, 2019). This would mean a change in favour of unsaturated fats, increasing the intake of polyunsaturated fatty acids, especially n-3 fatty acids at the expense of n-6 (FAO, 2010). Hence, it is necessary to try new strategies to ensure the nutritional quality of meat products but without losing the properties usually linked to this type of product and their acceptability (Heck et al., 2017). This not a simple task since fat plays an important role in the technological and sensory quality of meat products (Gómez & Lorenzo, 2013; de Oliveira Fagundes et al., 2017).

Olive oil is characterized by low saturated fatty acid content (<23%) and high unsaturated fatty acids in its composition (>71%), being oleic the major fatty acid (>63%) (Gavahian et al., 2019). This fatty acid has beneficial properties for human health, associated with reducing the risk of cardiovascular diseases (WHO, 2003). In addition, the presence in its composition of important amounts of tocopherols, especially α-tocopherol, and polyphenols makes it a perfect substitute for animal fat in meat products, even improving their shelf-life (Domínguez, Agregán, Gonçalves, & Lorenzo, 2016; Domínguez, Pateiro, Agregán, & Lorenzo, 2017a; Reddy, Jayathilakan, & Pandey, 2015). In the same way, the consumption of PUFA, especially n-3 fatty acids such as □-linolenic, has a high impact on health related to its cardiovascular benefits (Nagy & Tiuca, 2017). Chia and linseed oils are between the oils that have a high content of these polyunsaturated fatty acids. Moreover, these oils offer promising

possibilities due to their technological properties, such as their emulsifying activity, and positive and relevant health-benefits (Ansorena, & Astiasarán, 2004; Pintado, Ruiz-Capillas, Jiménez-Colmenero, Carmona, & Herrero, 2015a).

Several studies assessed the replacement of animal fat added to meat products by different sources of vegetable oils (Domínguez et al., 2017a; Heck et al., 2017; Pires, dos Santos, Barros, & Trindade, 2019). The challenge is to find an oil capable of promoting the improvement of the fatty acid profile without affecting the characteristics of the final product. Vegetable oils (such as chia, olive and linseed oil) can improve the profile of fatty acids by the larger amount of unsaturated fatty acids, characterized by their health benefits (Domínguez, Pateiro, Munekata, Campagnol, & Lorenzo, 2017b). However, their addition in meat products could compromise the physical and chemical characteristics of the product due to their substantially different characteristics such as taste, color, and consistency. In addition, their higher contents of unsaturated fatty acids could lead to rancidity, since these fatty acids are more rapidly oxidized.

Despite the beneficial effects of the presence of oleic or □-linolenic in reformulated meat products, this work intends to evaluate the influence that the substitution of animal fat with linseed, olive and chia oils could have on the structural and textural properties, and consequently on the sensorial characteristics and oxidative stability of cooked lamb sausages during their self-life.

2. MATERIAL AND METHODS

2.1. Raw materials, chemicals and reagentes

The raw materials such as lean lamb meat from shoulder, lamb heart and pork jowl were obtained from a local market, while commercial frankfurter sausage mix (Ceylamix®: 073 Frankfurter sausages) and sodium caseinate were obtained from Ceylan (Valencia, Spain). On the other hand, FAME 37 Mix, cis-vaccenic acid, trans-vaccenic acid and docosapentaenoic acid (> 97%) were supplied from Sigma-Aldrich (Madrid, Spain) and CLA from Matreya (Brockville, Canada). Hydrochloric (36,8-38%,

ACS BASIC) and sulphuric acid (95-98%, ACS BASIC), ethyl acetate (ACS BASIC), ethanol and methanol (reagent grade; 99.9%), sodium chloride (reagent grade) and trichloroacetic acid (reagent grade) were obtained from Scharlau (Barcelona, Spain). 4,6-dihidroxy-2-mercaptopyrimidine or thiobarbituric acid (TBA) (reagent grade; 98%) and 1,1-3,3 tetraetoxipropane (TEP) was obtained from Acros organics (Madrid, Spain). Bovine serum albumin was supplied by Calbiochem (Merck; Darmstadt, Germany), guanidine hydrochloride by Panreac (AppliChem ITW Reagents; Darmstadt, Germany) and 2,4-dinitrophenyl hydrazine (DNPH) by Fluorochem (Derbyshire, United Kingdom).

2.2. Cooked sausages manufacture

Four treatments of cooked lamb sausages, with the total substitution of animal fat by olive, linseed and chia oils were elaborated in the pilot plant of the Meat Technology Center of Galicia. A total of 160 sausages with a weigh of 25 g were manufactured (4 treatments x 4 sampling points x 5 samples for each sampling point x 2 different processing batches). Four batches were assessed: a control (CONT; with pork fat); and three with 100% fat replaced by commercial vegetable oils: chia (CHIA), linseed (LINS), and olive (OLIV). The ingredients used in the formulations were fat/oil emulsion (459.5 g backfat or oil, 92.2 g sodium caseinate, and 459.5 g water), pork jowl (1.17 kg), lean lamb (0.53 kg), lamb heart (0.64 kg), ice (1.11 kg), sodium caseinate (31.9 g), and commercial mix for frankfurter sausages. The fat substitution by the oils was carried out by weight.

Base fat (CONT) or oils (chia, linseed, or olive) were pre-emulsified. Water and sodium caseinate were homogenized in an Ultraturrax T25 basic (IKA-Werke, Staufen, Germany) during 2 min in a proportion of 5:1 (approximately 60 °C). Then five parts of oil or pork backfat (previously cut) were incorporated and emulsified during 3 min. The emulsified mass was cooled to ambient temperature (around 25 °C). Lamb meat, heart and jowl were chopped into a cutter (Cutter K30, Valencia, Spain) during 1 min. The fat/oils emulsion, ice, sodium caseinate, and commercial frankfurter sausage mix (Ceylamix®: 073 Frankfurter sausages, Valencia, Spain) were incorporated to the lamb meat mass, mixed for 5 min, and were embedded in a 22 mm caliber artificial casing.

The sausages were vacuum packed and cooked in a water bath (90 °C/10 min). The artificial casing was eliminated, and then, the sausages were packed again and pasteurized (85 °C/15 min), the samples were stored at 2 °C for 90 days. Samples were manufactured with the same ingredients and formulation in two batches separated two months in time.

2.3. Proximate composition

The chemical analysis (moisture, protein and ash content) was assessed according to the ISO recommended standards 1442:1997 (ISO, 1997), 937:1978 (ISO, 1978), and 936:1998 (ISO, 1998). Fat was extracted in an extractor Ankom XT10 (ANKOM Technology Corp., Macedon, NY, USA) following the A.O.C.S. Official Procedure Am 5-04 (AOCS, 2005).

2.4. Fatty acid composition

For fatty acid analysis, total fat was extracted from 10 g of sample (Bligh & Dyer, 1959). The fatty acid were transesterified according to Domínguez, Crecente, Borrajo, Agregán, & Lorenzo (2015) procedure with modifications proposed by Barros et al. (2020): For the fatty acids transesterification, twenty milligrams of extracted fat dissolved in 1 mL of toluene were mixed with 2 mL of a sodium methoxide (0.5 N) solution, vortexed during 10 seconds and allowed to stand for 15 minutes at room temperature. Then 4 mL of a H2SO4 solution (10% of H2SO4 in methanol) was added, vortexed for a few seconds and vortexed again before adding 2 mL of saturated sodium bicarbonate solution. For the extraction of fatty acid methyl esters, 1 mL of hexane was added to the samples, vortexed for 10 seconds and the organic phase was then transferred to an appropriate GC vial.

Separation and quantification of FAMEs were carried out using a gas chromatograph (GC-Agilent 7890B, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and PAL RTC-120 autosampler. One microliter of sample was injected in split mode (1:50). The injector was maintained at 250°C and 64.2 mL/min of total flow. For the separation of FAMEs, a DB-23 fused silica

capillary column (60 m, 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies, Santa Clara, CA, USA) was used. Chromatographic conditions were as follows: initial oven temperature of 50°C (held for 1 min), first ramp at 25°C/min to 175°C, second ramp at 4°C/min to 230°C (held for 5 min) and third ramp at 4°C/min to a final temperature of 240°C (held for 2.75 min). Helium was used as carrier gas at a constant flow-rate of 1.2 mL/min, with the column head pressure set at 22.9 psi. The FID detector was maintained at 280°C, while the operational flows were set as 40 mL/min of H2, 450 mL/min of air and 30 mL/min of makeup flow. The total time for chromatographic analysis was 30 minutes. Data acquisition and equipment control was carried out using the software MassHunter GC/MS Acquisition B.07.05.2479 (Agilent Technologies, Santa Clara, CA, USA), while the data analysis was carried out with the software MassHunter Quantitative Analysis B.07.01. Individual FAMEs were identified by comparing their retention times with those of authenticated standards (FAME Mix -37 components-; docosapentaenoic acid; trans-vaccenic acid; cis-vaccenic acid and CLA) and the results were expressed as g/100 g of total fatty acids identified.

The total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid contents were calculated. To assess the nutritional properties of fat the ratios PUFA/SFA and n-6/n-3, and the indexes of atherogenicity (IA) and thrombogenicity (IT) (Ulbricht & Southgate, 1991) were determined.

2.5. Color parameters and pH

The pH was determinated in the sausages using a digital pH-meter (Hanna Instruments, Eibar, Spain) equipped with a penetration probe. On the other hand, color parameters were assessed in the CIELAB space (lightness, L*; redness, a*; and yellowness, b*) using a portable colorimeter (CR-600d, Minolta Co. Ltd., Osaka, Japan). The measurements were taken on the surface of the sausage after 30 min exposure to the atmosphere at six different points.

The total colour difference (ΔE*) between cooked lamb sausages at day 0 and the other days of storage was calculated using the next formula (Yudd & Wyszcki, 1975):

$$\Delta E^* = [(L^*_{30-90} - L^*_0)^2 + (a^*_{30-90} - a^*_0)^2 + (b^*_{30-90} - b^*_0)^2]^{1/2}$$

2.6. Lipid and protein oxidation

The evaluation of lipid stability was assessed by measuring of Thiobarbituric Acid Reacting Substances (TBARs) values following the method proposed by Vyncke (1975). Briefly, a cooked lamb sausage sample (2 g) was dispersed in 5% trichloroacetic acid (10 mL) and homogenized in an Ultra-Turrax (IkaT25 basic, Staufen, Germany) for 2 min. The homogenate was maintained at −10 °C for 10 min and centrifuged at 2360 g for 10 min. The supernatant was filtered through a Whatman No. 1 filter paper. The filtrate (5 mL) was reacted with a 0.02 M TBA solution (5 mL) and incubated in a water bath at 96 °C for 40 min. The absorbance was measured at 532 nm. The TBARS value was calculated from a standard curve of malonaldehyde with TEP and expressed as mg malonaldehyde (MDA) per kg of the sample.

Protein oxidation was measured according to the method outlined by Oliver, Ahn, Moerman, Goldstein, & Stadtman (1987) with modifications (Vuorela et al., 2005). Two different measurements (carbonyl and protein quantification) were made for protein oxidation. 100 µL of homogenate obtained from the homogenisation of samples (2.5 g) with 20 mL of 0.6 M NaCl solution was treated with 1 mL of 10% trichloroacetic acid and centrifuged for 5 min at 5000 g. The supernatant was derivatized for carbonyl quantification with 1 mL of 2 M HCl with 0.2% 2,4-dinitrophenyl hydrazine (DNPH), while for protein quantification 1 mL of 2 M HCl was added. The pellet obtained was washed with 1 mL of ethanol/ethyl acetate (1:1) three times, and then dissolved in 1.5 mL of 20 mM sodium phosphate buffer with 6 M guanidine hydrochloride. Carbonyls and protein concentration were measured spectrophotometrically at 370 and 280 nm, respectively. The protein concentration was calculated using bovine serum albumin as standard, and the protein concentrations were determined according to a standard curve. The results were expressed as nmol of carbonyl/mg of protein.

2.7. Analysis of volatile compounds

The extraction of the volatile compounds was performed using solid-phase microextraction (SPME), following the method described by Dominguez et al. (2019a). For headspace SPME (HS-SPME) extraction, 1 g of each sample, after being ground using a commercial grinder, was placed in a 20 mL vial. The conditioning, extraction

and injection of the samples were carried out with a PAL RTC 120 auto sampler (CTC Analytics AG, Zwingen, Switzerland). The extractions were carried out at 37 ºC for 30 min, after equilibration of the samples for 15 min at the temperature used for extraction, ensuring a homogeneous temperature for sample and headspace. Once sampling was finished, the fibre was transferred to the injection port of the gas chromatograph–mass spectrometer (GC–MS) system. A 7890B gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a 5977B MSD mass selective detector (Agilent Technologies) and a DB-624 capillary column (30 m, 0.25 mm i.d., 1.4 □m film thickness) (J&W Scientific, Folsom, USA) was used for volatile analysis. Compounds were identified by comparing their mass spectra with those contained in the NIST14 (National Institute of Standards and Technology, Gaithersburg, USA) library (match factor > 85%), and/or by comparing their mass spectra and retention time with authentic standards. The results are expressed as area units (AU) × 104/g of sample.

2.8. Texture profile analysis

The texture profile analysis (TPA) test was performed using a texturometer (TA.XT.plus of Stable MicroSystems, Vienna Court, UK) equipped with a probe of 19.85 cm² of surface contact linked to a 30 kg cell. Samples measuring 2 cm in length were compressed twice to 50% with a compression speed of 1 mm/s to determine the hardness (N), springiness (mm), cohesiveness and chewiness (N.mm) of the cooked lamb sausages.

2.9. Sensory analysis

A total of 69 consumers selected from Ourense (Spain) carried out the sensory analysis. An acceptance test was realized in individual cabins under white and red lights, for the attributes evaluated in raw and cooked samples, to determine whether the panelist liked or disliked the cooked lamb sausages manufactured with fat substitution of animal fat with linseed, olive and chia oils. The panelists assessed the following sensory parameters in raw sausages (color, surface discoloration, and odor at 0, 30, 60, and 90 days of storage) or cooked (odor and flavor, only on day 0) using

a five-point scale (1 = excellent, 5 = not acceptable) proposed by Lago et al. (2017). Moreover, the panelists scored the samples according to their preference. Cooked sausages were cooked in a furnace (Rational CombiMaster®Plus CMP61, Landsberg am Lech, Germany) equipped with a central temperature probe until reaching an internal temperature of 70 °C. The samples were served to panelists on plastic plates encoded with random three-digit in a sequential monadic way, following a Latin square design (MacFie, Bratchell, Greenhoff & Vallis, 1989). Water and unsalted toasted bread were used at the beginning of sessions and between samples to clean the palate and remove residual flavours.

2.10. Statistical analysis

A completely randomized design was applied with four batches (CONT, CHIA, LINS, OLIV) and five repetitions. A total of 40 sausages were assessed in each point: five sausage samples for each batch × four batches × two different processing batches (CONT, CHIA, LINS, and OLIV). Previously normal distribution and variance homogeneity were tested (Shapiro-Wilk). The data were submitted to a two-way analysis of variance (ANOVA; with treatment and storage time as a fixed effect for the variables evaluated in time) or a one-way analysis of variance (for the variables evaluated only at day 0), followed by Tukey´s test when the ANOVA was significant ($P<0.05$). For sensory data, Friedman's two-way analysis of variance by ranks test was applied, followed by the Wilcoxon test with a Bonferroni correction for multiple pairwise comparisons when a significant difference was detected between the treatments ($P<0.05$). Statistical analyses were performed using SAS (version 9.4, NC, USA).

3. RESULTS AND DISCUSSION

3.1. Chemical composition

The replacement of backfat by vegetable oils had a significant effect ($P<0.001$) on the proximal composition of lamb cooked sausages (Table 1), presenting mean values of 53.21-55.55% for moisture, 20.08-24.95% for fat, 18.27-18.73% for protein,

and 2.77-2.99% for ash. CONT and OLIV sausages presented the highest values for moisture (55.55 and 54.98%), fat (23.77 and 24.95%), and protein (8.73 and 8.71%), respectively; while the ash content was higher only in CONT sausages (2.99%). These outcomes disagree with the data found by Monteiro et al. (2017), who did not found differences in raw sausage treatments with different levels of pork fat substitution with canola oil. However, according to other studies a significant decrease of fat content could be observed when animal fat was replaced by fish or vegetable oils (Domínguez et al., 2017a; Selani et al., 2016).

Table 1. Effect of the replacement of backfat by vegetable oils (chia, linseed and olive) on proximate composition and fatty acid profile of cooked lamb sausages (mean values ± standard deviation).

	CONT (n=10)	CHIA (n=10)	LINS (n=10)	OLIV (n=10)	Sig.
Composition (%)					
Moisture	55.55±0.53^{a}	53.21±0.58^{b}	53.98±0.23^{b}	54.98±0.33^{a}	***
Fat	23.77±0.65^{a}	20.08±0.54^{b}	20.38±0.52^{b}	24.95±0.11^{a}	***
Protein	18.73±0.31^{a}	18.33±0.10^{b}	18.27±0.11^{b}	18.71±0.14^{a}	***
Ash	2.99±0.05^{a}	2.86±0.04^{b}	2.85b±0.04^{c}	2.77±0.03^{c}	***
Fatty acid profile					
C14:0	1.32±0.00^{a}	0.98±0.00^{b}	0.98±0.00^{b}	0.94±0.00^{c}	***
C14:1n-5	0.02±0.00^{a}	0.01±0.00^{bc}	0.01±0.00^{c}	0.01±0.00^{b}	***
C15:0	0.08±0.01^{a}	0.06±0.00^{b}	0.06±0.00^{c}	0.06±0.00^{b}	***
C16:0	22.94±0.07^{a}	18.36±0.07^{d}	18.71±0.10^{c}	19.65±0.11^{b}	***
C16:1n-7	1.69±0.02^{a}	1.19±0.00^{d}	1.31±0.01^{c}	1.62±0.00^{b}	***
C17:0	0.46±0.00^{a}	0.35±0.00^{c}	0.36±0.00^{b}	0.36±0.00^{b}	***
C17:1n-7	0.33±0.00^{a}	0.24±0.00^{d}	0.26±0.00^{c}	0.30±0.00^{b}	***
C18:0	13.82±0.07^{a}	11.04±0.05^{c}	11.53±0.04^{b}	10.42±0.10^{d}	***
9t-C18:1	0.57±0.01^{a}	0.43±0.00^{b}	0.40±0.00^{c}	0.41±0.00^{c}	***
C18:1n-9	38.37±0.19^{b}	28.58±0.14^{d}	33.11±0.11^{c}	49.60±0.40^{a}	***
C18:1n-7	2.31±0.02^{a}	1.79±0.04^{b}	1.83±0.03^{b}	2.34±0.00^{a}	***

C18:2n-6	14.32±0.09^{b}	15.52±0.09^{a}	13.58±0.05^{c}	11.28±0.17^{d}	***
9t,11t-C18:2	0.03±0.00^{a}	0.02±0.00^{b}	0.02±0.00^{c}	0.02±0.00^{bc}	***
C20:0	0.26±0.00^{c}	0.27±0.00^{b}	0.24±0.00^{d}	0.32±0.00^{a}	***
C18:3n-6	nd	0.09±0.00	0.08±0.00	nd	***
C20:1n-9	0.86±0.00^{a}	0.61±0.01^{b}	0.59±0.00^{c}	0.60±0.00^{c}	***
C18:3n-3	0.60±0.00^{c}	18.91±0.01^{a}	15.49±0.12^{b}	0.65±0.00^{c}	***
C21:0	0.08±0.00^{a}	0.08±0.00^{a}	0.07±0.00^{b}	0.07±0.00^{b}	***
C20:2n-6	0.63±0.00^{a}	0.44±0.00^{b}	0.39±0.00^{c}	0.37±0.00^{d}	***
C22:0	0.04±0.00^{a}	0.03±0.00^{b}	0.03±0.00^{b}	0.02±0.00^{c}	***
C20:3n-6	0.10±0.00^{a}	0.07±0.00^{c}	0.07±0.00^{b}	0.06±0.00^{c}	***
C22:1n-9	0.01±0.00^{a}	0.01±0.00^{b}	0.01±0.00^{bc}	0.01±0.00^{c}	***
C20:3n-3	0.08±0.00^{a}	0.07±0.00^{b}	0.07±0.00^{b}	0.05±0.00^{c}	***
C23:0	0.33±0.00^{a}	0.26±0.00^{b}	0.24±0.00^{c}	0.26±0.00^{b}	***
C20:4n-6	0.33±0.00^{a}	0.26±0.00^{b}	0.24±0.00^{c}	0.26±0.00^{b}	***
C20:5n-3	0.01±0.00^{b}	0.02±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	***
C22:5n-6	0.11±0.00^{a}	0.08±0.00^{b}	0.07±0.00^{c}	0.07±0.00^{c}	***
C22:5n-3	0.07±0.00^{a}	0.05±0.00^{b}	0.05±0.00^{c}	0.05±0.00^{c}	***
C22:6n-3	0.03±0.00^{a}	0.02±0.00^{b}	0.02±0.00^{c}	0.02±0.00^{c}	***
SFA	39.33±0.13^{a}	31.44±0.01^{c}	32.21±0.14^{b}	32.10±0.23^{b}	***
MUFA	44.20±0.10^{b}	32.87±0.11^{d}	37.54±0.10^{c}	54.90±0.37^{a}	***
PUFA	16.31±0.11^{c}	35.54±0.08^{a}	30.10±0.12^{b}	12.87±0.16^{d}	***
PUFA/SFA	0.41±0.01^{c}	1.13±0.00^{a}	0.93±0.00^{b}	0.40±0.00^{d}	***
n-3	0.80±0.12^{c}	19.08±0.01^{a}	15.66±0.13^{b}	0.80±0.01^{c}	***
n-6	15.51±0.06^{b}	16.47±0.01^{a}	14.45±0.06^{c}	12.07±0.017^{d}	***
n-6/n-3	19.38±0.00^{a}	0.86±0.01^{c}	0.92±0.00^{c}	15.14±0.42^{b}	***
AI	0.47±0.00^{a}	0.32±0.00^{d}	0.33±0.00^{c}	0.34±0.00^{b}	***
TI	1.18±0.00^{a}	0.37±0.00^{d}	0.42±0.00^{c}	0.86±0.00^{b}	***

$^{a-d}$ Mean values in the same row with different lowercase letters indicate significant difference. nd: not detected; Sig.: significance; *** P<0.001.

3.2. Fatty acid profile

As expected, we found significant differences (P<0.001) in the fatty acid profile of the reformulated sausages (Table 1). The MUFA were the main fatty acids in the CONT, LINS, and OLIV groups (44.20%, 37.54%, and 54.90%, respectively). The

CHIA batch presented PUFA as major fatty acids (35.54%). Among the saturated fatty acids, palmitic acid (C16:0) was the majority in all the batches ranging from 18.46% to 22.95%; showing highest values in the CONT group. Oleic acid (C18:1n-9c) was the predominant MUFA, ranging from 28.58% to 49.60% for the CHIA and OLIV groups, respectively. Regarding PUFA, linoleic (C18:2n-6c) acid was prevalent in CONT (14.32%) and OLIV (11.28%), while linolenic acid (C18:3n-3) was predominant in CHIA (18.91%) and LINS (15.49%), indicating an enhancement in the fatty acid composition of these treatments in comparison with CONT group. This finding is in agreement with data found by Heck et al. (2017), who noticed an increase in the PUFA content in low-fat burgers when the pork fat was changed by chia oil.

Concerning the nutritional aspect, the PUFA/SFA ranged from 0.40 to 1.13; presenting higher value in the CHIA group. The PUFA/SFA ratio is an important parameter to determinate the nutritional characteristics of the lipid composition in foods. FAO (2010) recommends a ratio above 0.85 for the human diet. In the present study, only the CHIA and LINS batches reached this value. This fact could be explained by the high n-3 amount found in these groups, also changing the n-6/n-3 ratio; unsurprisingly, the lowest values of this ratio were observed in CHIA and LINS (0.86 and 0.92, respectively) while the CONT and OLIV groups showed higher values (19.38 and 15.14, respectively) than the maximum level (4.00) proposed by FAO (2010).

The addition of vegetable oils had a significant effect on the AI and TI ($P<0.001$) ranging from 0.32 to 0.47 and from 0.37 to 0.42, respectively, with higher values observed in the CONT treatment and the lower values for the CHIA group, evidencing the enhancement in the lipid composition reached with the replacement of fat by vegetable oils.

These outcomes agree with those obtained by other authors who reported a decrease of AI and TI using vegetable oils such as echium and olive oil as fat replacers in bologna and frankfurter type sausage, respectively (Domínguez et al., 2017b; Pires et al., 2019). Similar results were also found in other meat products containing healthy oil emulsion as a fat substitute (Domínguez et al., 2016; Heck et al., 2017; Selani et al., 2016).

3.3. pH and instrumental color

The color parameters and pH values of the reformulated sausages during refrigerated period is presented in Table 2. Throughout the whole display, the pH of the CONT treatment decreased (6.18-6.12), whereas, in the LINS and OLIV groups, the pH increased (6.14-6.22 and 6.15-6.24, respectively). In CHIA batch, the pH did not display significant difference ($P>0.05$) between the beginning and the end of storage period. The higher values among the batches (6.22 and 6.24 on day 90) were found in LINS and OLIV group, respectively. These outcomes agree with data reported by other authors (Seo, Yum, Kim, Jeong, & Yang, 2016) who observed similar values in frankfurters. The changes in the pH indicated that the storage time and addition of oils affect the pH. These outcomes are in agreement with data reported by Domínguez et al. (2016) who observed that pH was significantly influenced by fat substitution with olive oil in pork pâté. Besides the significant differences observed among treatment, there is no a clear trend and the values were similar.

Table 2. Effect of chia, linseed and olive oils as backfat replacers on pH and color parameters of cooked lamb sausage during refrigerated storage (mean values ± standard deviation).

		Treatment				
	Days	CONT (n=40)	CHIA (n=40)	LINS (n=40)	OLIV (n=40)	Sig.
pH	0	6.18 ± 0.02^{Aa}	6.19 ± 0.01^{Aa}	6.14 ± 0.04^{Bb}	6.15 ± 0.01^{ABb}	*
	30	6.18 ± 0.03^{ab}	6.19 ± 0.03^{a}	6.19 ± 0.02^{a}	6.22 ± 0.01^{a}	ns
	60	6.11 ± 0.01^{Bc}	6.11 ± 0.01^{Bb}	6.13 ± 0.01^{Bb}	6.16 ± 0.03^{Ab}	***
	90	6.12 ± 0.07^{Bbc}	6.21 ± 0.06^{ABa}	6.22 ± 0.03^{Aa}	6.24 ± 0.02^{Aa}	*
	Sig.	**	**	***	***	
L*	0	67.25 ± 0.72^{C}	70.34 ± 0.31^{Ab}	69.62 ± 0.72^{ABc}	68.86 ± 0.27^{Bb}	***
	30	65.90 ± 0.53^{C}	70.10 ± 0.74^{Ab}	70.81 ± 0.56^{Abc}	68.84 ± 0.57^{Bb}	***
	60	67.16 ± 1.24^{B}	72.39 ± 1.12^{Aa}	73.00 ± 0.41^{Aa}	71.75 ± 0.64^{Aa}	***
	90	66.46 ± 1.31^{B}	72.29 ± 0.45^{Aa}	72.06 ± 1.40^{Aab}	71.16 ± 0.66^{Aa}	***
	Sig.	ns	***	***	***	
a*	0	9.67 ± 0.50^{A}	8.00 ± 0.17^{B}	8.31 ± 0.58^{B}	8.11 ± 0.41^{B}	***
	30	9.26 ± 0.55^{A}	7.95 ± 0.16^{B}	7.93 ± 0.53^{B}	8.19 ± 0.42^{B}	***
	60	9.90 ± 0.28^{A}	7.66 ± 0.33^{B}	7.79 ± 0.43^{B}	7.68 ± 0.30^{B}	***
	90	9.64 ± 0.24^{A}	7.78 ± 0.22^{B}	8.10 ± 0.53^{B}	7.76 ± 0.17^{B}	***
	Sig.	ns	ns	ns	ns	
b*	0	16.84 ± 0.65^{B}	16.09 ± 0.17^{Bb}	17.94 ± 0.37^{Ab}	18.65 ± 0.58^{Ab}	***

	30	17.40 ± 0.66^{B}	16.74 ± 0.43^{Bb}	17.57 ± 0.26^{Bb}	18.73 ± 0.49^{Ab}	***
	60	17.75 ± 0.62^{C}	17.62 ± 0.64^{Ca}	18.98 ± 0.32^{Ba}	19.95 ± 0.35^{Aa}	***
	90	18.16 ± 0.98^{BC}	17.75 ± 0.54^{Ca}	19.28 ± 0.17^{ABa}	19.96 ± 0.84^{Aa}	***
	Sig.	ns	***	***	**	
ΔE*	0-30	2.23 ± 0.61^{Bb}	1.03 ± 0.48^{Aa}	2.08 ± 0.65^{Ba}	0.71 ± 0.24^{Aa}	***
	0-60	1.30 ± 0.38^{Aa}	3.13 ± 0.46^{Bb}	3.64 ± 0.54^{Bb}	3.34 ± 0.46^{Bb}	***
	0-90	2.65 ± 0.50^{b}	2.62 ± 0.42^{b}	2.48 ± 0.65^{a}	2.90 ± 0.44^{b}	ns
	Sig.	**	***	**	***	

$^{A-C}$ Mean values in the same row (different treatment in same day) with different capital letters indicate significant difference; $^{a-c}$ Mean values in the same column (same treatment in different days) with different lowercase letters indicate significant difference. Sig.: significance; n.s.: not significant;*P<0.05; ** P<0.01; *** P<0.001.

Instrumental color of the sausages was significantly influenced by oil incorporation. Regarding the L* values, the CONT group was not affected over time, presenting the lowest values during the whole display. The backfat replacers (CHIA, LINS, and OLIV treatments) rose the lightness of the sausages during the refrigerated period compared to CONT group ($P<0.05$), presenting values of 70.34, 69, 62, and 68.86 versus 67.25 at day 0 and 72.29, 72.06, and 71.16 versus 66.46 at day 90, respectively. On the other hand, for the a* values, no differences were found over time, but presented differences among the batches, since the CONT group differed ($P<0.05$) from other ones presenting the highest values at the beginning and at the end of storage (9.67 and 9.64, respectively). These results are in agreement with data reposted by Pires et al. (2019), who reported a reduction in the a* values in bologna sausage formulated with Echium oil. Over storage, reformulated sausages presented a rise in the b* values ($P<0.05$) with the highest values observed in the OLIV group, ranging from 18.65 to 19.96, while in the CONT treatment no difference was noticed in the b* values ($P>0.05$) during storage time.

In general, the differences found in color parameters were due to the replacement of animal fat by olive, chia and linseed oil emulsions, which have different compositions and consequently different tones of colour. Moreover, the oil globules diameter of the emulsions is smaller than the animal fat globules, which provides greater light reflection, thus increase L* values (Poyato, Ansorena, Berasategi, Navarro-Blasco, & Astiasarán, 2014). Moreover, color shades that these oils present could justify the increase in b*, since oils have a yellowish coloration while the pork jowl has a whitish colour. The results found in this work are in agreement with those found by other authors in meat products reformulated with olive, chia and linseed oils (Gómez, Sarriés, Ibañez, & Beriain, 2018; Heck et al., 2019).

These differences were also observed in ΔE* values during refrigerated storage. As commented before, these variations between cooked lamb sausages could be due to the existing differences in their fatty acid composition, which are expected to influence their susceptibility to oxidative deterioration during their shelf-life (Estévez & Cava, 2004). In fact, color changes during refrigerated storage could be associated to oxidation processes (Estévez, Ventanas, & Cava, 2006). ΔE*0–30 values showed that CHIA, LINS and OLIV possessed the lowest colour differences during storage (2.08, 1.03 and 0.71 vs. 2.23 for LINS, CHIA and OLIV vs. CONT, respectively; Table 2), thus

presenting a greater oxidative stability compared to CON samples. These colour differences could be due to the discoloration of the product as a result of oxidation processes during the storage period. In contrast, reformulated cooked lamb sausages displayed ΔE*0–60 values higher than CONT (ΔE* values higher than 3.00 vs. 1.30, respectively), being physical changes or modifications in sample composition the main responsible. However, at the end of storage the replacement did not show a significant effect on ΔE* values. These values found were near to the values that could be noticeable by the consumer (ΔE*>2; Francis & Clydesdale, 1975). This is consistent with the results found in the sensory evaluation of the products, where no significant differences were observed in color among batches and during the storage time showing acceptable levels in all batches/time.

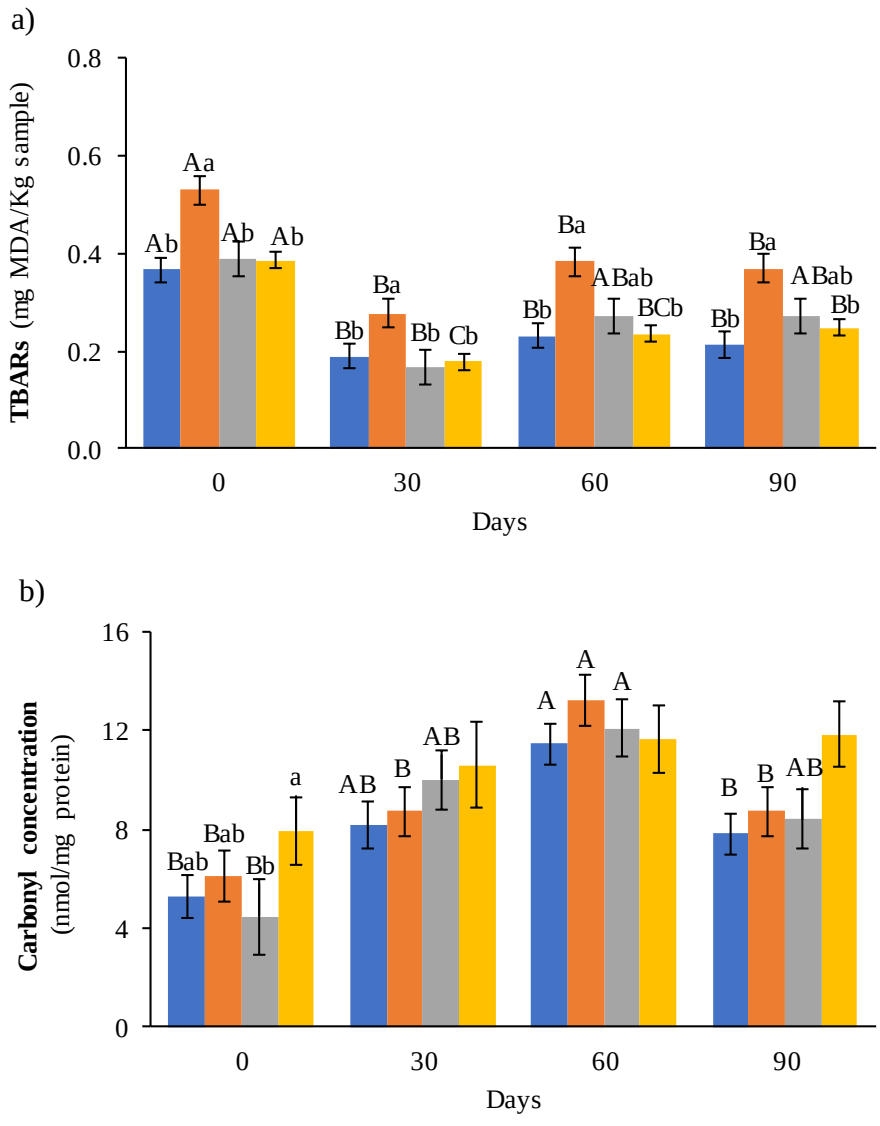
3.4. Lipid and protein oxidation

The effect of fat replacement by vegetable oils on oxidative stability is displayed in Fig. 1. The MDA content had the same behavior in all the treatments over time; decreased between day 0 and day 6 and remained stable thereafter ($P<0.05$). All the treatments presented TBARs values lower than the limit of acceptability of 2 mg MDA/kg (Greene & Cumuze, 1982). Among the batches, only the CHIA group was different from the CONT treatment ($P<0.05$) showing the highest values over time, ranging between 0.53 mg MDA/kg at beginning and 0.37 mg MDA/kg at the end of storage. This result in reformulated sausages with chia was expected due to its higher PUFA levels compared to CONT batch and agrees with data found by Pintado et al. (2015b). Nevertheless, these values are considered very low for 90 days of storage, even below the TBARS values (higher than 0.6 mg MDA/kg) considered as deterioration level of rancid flavour in meat products by Georgantelis, Blekas, Katikou, Ambrosiadis, & Fletouris (2007).

Lipid and heme-protein oxidation in meat occurs in a concurrent manner, so that the presence of lipid oxidation products could also favour heme-protein oxidation (Chaijan, 2008; Dominguez et al., 2019b). In this way, in the present way the fat replacement affected the protein oxidation most notably. In the CONT, CHIA, and LINS groups, the carbonyl content increased until day 60 when they reached their highest

values (11.48 nmol/mg, 13.24 nmol/mg, and 11.12 nmol/mg, respectively). The OLIV batch showed no difference over time; however, it had the highest values at the beginning and the end of the cold storage (7.96 nmol/mg and 11.87 nmol/mg, respectively). Carvalho et al. (2019) also reported an increase in the carbonyl levels in the lamb burgers with fat substitution by chia oil during refrigerated storage.

Figure 1. Evolution of TBARs values (a) and total carbonyl content (b) in cooked lamb sausages manufactured with backfat, chia, linseed, or olive oils during storage (■ CON, ■ CHIA, ■ LINS, ■ OLIV). Error bars corresponding to standard error. Different lowercase letters indicate a significant difference between the treatments and different capital letters indicate a significant difference in different days.



3.5. Analysis of volatile compounds

Volatile compounds profile from lamb sausages, assessed at the beginning and end of the display, are presented in Table 3. Examination of the volatile compounds in lamb sausage demonstrated that the major chemical families were aldehydes, ketones, and alcohols. This result corroborates the volatile compounds of lamb burger (Carvalho et al., 2019). In addition, Bravo-Lamas, Barron, Farmer, & Aldai (2018) reported that aldehydes, ketones, and alcohols are mainly produced from lipid oxidation reactions in cooked lamb burger.

Table 3. Volatile compounds (mean values ± standard deviation) in cooked lamb sausages prepared with chia, linseed and olive oils as replacer of backfat (AU × 10^4/g of sample)

	m/z	LRI	Day	Treatments				Sig.
				CONT (n=20)	CHIA (n=20)	LINS (n=20)	OLIV (n=20)	
Aldehydes								
2-Heptenal, (E)-	83	1068	0	2.26±0.23^{a}	1.92±0.42^{a}	1.22±0.31^{b}	0.46±0.07^{c}	***
			90	4.98±1.87^{ab}	6.17±0.52^{ab}	1.01±0.34^{b}	27.71±2.42^{a}	*
			Sig.	*	***	ns	*	
2-Octenal, (E)-	112	833	0	2.47±1.30	1.69±0.70	3.01±0.62	2.21±0.32	ns
			90	2.33±1.26	3.59±0.88	3.18±1.25	4.27±1.34	ns
			Sig.	ns	**	ns	*	
Pentanal	57	728	0	6.70±2.45^{b}	112.66±21.18^{a}	6.12±0.53^{b}	6.37±0.58^{b}	***
			90	2.35±0.69^{c}	231.48±11.07^{a}	35.35±8.91^{b}	33.27±1.57^{b}	***
			Sig.	**	***	***	**	
Hexanal	56	865	0	81.15±5.21^{a}	73.80±19.67^{a}	25.87±12.88^{b}	0.17±0.05^{b}	***
			90	13.93±1.07^{bc}	83.64±11.10^{a}	1.36±0.19^{c}	57.81±15.26^{ab}	***
			Sig.	***	ns	ns	ns	
Heptanal	70	974	0	8.10±0.89^{b}	4.51±1.21^{bc}	2.02±0.1.65^{c}	19.88±5.77^{a}	***
			90	2.35±1.68^{c}	5.36±0.33^{b}	0.14±0.09^{d}	11.26±0.79^{a}	***
			Sig.	***	ns	ns	*	

Nonanal	57	1148	0	6.18±2.13^{a}	3.39±0.25^{b}	3.90±1.08^{b}	1.12±0.71^{c}	***
			90	3.27±1.17^{b}	3.32±0.49^{b}	3.25±1.08^{b}	13.97±6.91^{a}	***
			Sig.	*	ns	ns	**	
Ketones								
2-Pentanone	86	720	0	0.23±0.11	0.41±0.15	0.24±0.10	0.39±0.30	ns
			90	0.80±0.39	0.66±0.26	0.68±0.23	1.52±1.04	ns
			Sig.	ns	ns	ns	ns	
2,3-Pentanedione	100	735	0	0.11±0.07^{c}	2.08±0.71^{b}	0.86±0.44^{bc}	3.80±1.30^{a}	***
			90	0.01±0.00	0.72±0.22	0.55±0.35	0.63±0.40	ns
			Sig.	ns	**	ns	**	
2-Heptanone	58	967	0	7.66±0.90	7.50±1.30	8.33±1.16	8.44±1.24	ns
			90	28.84±4.74^{a}	12.10±0.67^{b}	15.38±5.27^{b}	14.68±4.36^{b}	***
			Sig.	***	***	*	*	
Alcohols								
1-Pentanol	55	847	0	6.39±0.81^{c}	8.30±1.57^{c}	12.04±1.93^{b}	18.88±2.19^{a}	***
			90	15.17±2.07	10.78±1.35	15.70±4.11	14.44±3.44	ns
			Sig.	***	*	ns	*	
1-Hexanol	56	969	0	2.80±0.38^{b}	9.08±1.83^{b}	54.61±14.69^{a}	51.86±4.21^{a}	***
			90	41.74±22.47^{a}	10.33±4.99^{b}	54.00±17.16^{a}	27.90±8.30^{ab}	**
			Sig.	***	ns	ns	***	
1-Octen-3-ol	57	1051	0	5.84±0.51^{b}	6.09±1.10^{b}	5.97±1.12^{b}	17.53±3.75^{a}	***

	90	10.25±2.55[b]	10.99±1.15[b]	10.88±1.42[b]	41.80±1871[a]	***
	Sig.	**	***	***	*	

[a-d] Mean values with different capital letters indicate significant difference between treatments; Sig.: significance; n.s.: not significant; * *P*<0.05; ** *P*<0.01; *** *P*<0.001; m/z: Quantification ion; LRI: Lineal Retention Index calculated for DB-624 capillary column (J&W scientific: 30m×0.25mm id, 1.4 µm film thickness) installed on a gas chromatograph equipped with a mass selective detector.

Regarding the aldehyde family, the CONT group displayed a significant decrease ($P<0.05$) in the levels of aldehydes studied over time (heptanal, nonanal, pentanal, and hexanal). Reformulated sausages showed an increase in the pentanal levels over time and the CHIA batch presented the higher value (231.48 AU x 104/g) at 90 days. According to Stetzer, Cadwallader, Sing, Mckeith, & Brewer (2008), pentanal is negatively correlated with the quality of taste, which could bring sensory damage. However, the differences aforementioned did not affect the odor of the sausages perceived by panelists in the sensory analysis (Fig. 2. D). Hexanal is predominant among the aldehyde family (Maggiolino et al., 2019) and the principal indicator of lipid oxidation. In our study, the hexanal level of the CONT group dropped over time (from 81.15 AU x 104/g to 13.93 AU x 104/g), and there were no differences in the reformulated sausages over time ($P>0.05$). This result could be linked to the low lipid oxidation in all treatments, evidenced by the TBARs values (Fig. 1 A).

The ketone group was also affected by fat replacement, 2,3-pentanedione differed significantly ($P<0.05$) among the treatments at day 0, with a higher value showed by the OLIV group (3.80 AU x 104/g). The 2-heptanone levels increased in all the batches over time ranging from 7.50 AU x 104/g to 8.44 AU x 104/g at day 0 and from 12.10 AU x 104/g to 28.84 AU x 104/g at the end of refrigerated period; since the highest value was found in the CONT group. These outcomes agree with Carvalho et al. (2019), who found an increase of 2-heptanone levels in the lamb burgers over time. No differences in the 2-pentanone amount were found between batches during the whole display. Ketones, along with aldehydes, are recognized as the predominant aromatic compounds derived of the fatty acid oxidation (Resconi, Escudero, & Campo, 2013).

Overall, within the alcohol family, there was a significant increase in 1-hexanol, 1-pentanol, and 1-octen-3-ol levels through the whole display. Among the groups, there was a significant difference in 1-hexanol, and 1-octen-3-ol levels, the higher values (54.00 AU x 104/g and 41.80 AU x 104/g) were found in the LINS and OLIV group at day 90, respectively. On the other hand, 1-pentanol did not differ among the batches at the end cold storage. These volatile compounds are main compounds found considered as marker of oxidative stability in meat products (Resconi et al., 2018).

3.6. Texture profile analysis

Examination of TPA revealed that the inclusion of vegetable oils had effect on hardness, springiness, chewiness, and cohesiveness (Table 3). In this way, sausages reformulated with chia oil presented the highest values for the parameters evaluated. Regarding hardness, the CHIA and OLIV groups had a linear increase over time from 13.01 N to 18.30 N and from 12.56 N to 15.60 N, respectively. A significant linear increase was also noticed in the chewiness values of the CHIA groups over time, ranging from 5.08 N/mm to 7.07 N/mm, which could explain the variation among the treatments at the end cold storage since the CHIA batch presented the highest values. These results are in accordance with those noticed by several authors who showed a rise in the TPA of the meat products with backfat replacement with vegetable oils (Afshari, Hosseini, Khaneghah, & Khaksar, 2017; Baek, Utama, Lee, An, & Lee, 2016). Therefore, the differences observed among the treatments can be attributed to the different characteristics of the emulsions used in the formulations of cooked lamb sausages. In addition, the protein oxidation found in the present study can lead to changes in gelation, viscosity, solubility, emulsification, texture, and juiciness (Baron, Kjaersgard, Jessen, & Jacobsen, 2007) and increase in the deformation compression force and hardness of the sausages.

Table 4. Effect of chia, linseed and olive oils as backfat replacers on texture profile analysis of cooked lamb sausage during refrigerated storage (mean values ± standard deviation).

		Treatment				
	Days	CONT (n=40)	CHIA (n=40)	LINS (n=40)	OLIV (n=40)	Sig.
Hardness (N)	0	13.09±0.09	13.01±0.08^{b}	13.36±0.18	12.56±0.07^{c}	ns
	30	13.65±0.04	13.25±0.02^{b}	13.96±0.14	13.82±0.04^{bc}	ns
	60	14.81±0.10	15.40±0.19^{b}	15.29±0.07	15.25±0.07^{ab}	ns
	90	14.82±0.14^{B}	18.30±0.14^{Aa}	15.41±0.17^{B}	15.60±0.12^{Ba}	*
	Sig.	ns	***	ns	***	
Springiness (mm)	0	0.71±0.02^{a}	0.74±0.02	0.73±0.01	0.72±0.03	ns
	30	0.67±0.02^{Bab}	0.72±0.01^{A}	0.72±0.02^{A}	0.75±0.01^{A}	***
	60	0.69±0.02^{Ba}	0.75±0.02^{A}	0.74±0.02^{A}	0.74±0.02^{A}	***
	90	0.63±0.03^{Bb}	0.72±0.02^{A}	0.71±0.04^{A}	0.72±0.02^{A}	***
	Sig.	*	ns	ns	ns	
Cohesiveness	0	0.50±0.04^{B}	0.53±0.03^{AB}	0.55±0.01^{AB}	0.57±0.02^{A}	*
	30	0.53±0.02	0.53±0.03	0.52±0.04	0.56±0.01	ns
	60	0.51±0.02	0.55±0.04	0.51±0.02	0.54±0.04	ns
	90	0.47±0.03	0.53±0.04	0.52±0.05	0.53±0.03	ns
	Sig.	ns	ns	ns	ns	
Chewiness (N.mm)	0	4.67±0.08	5.08±0.02^{b}	5.34±0.08	5.18±0.04	ns

30	4.84±0.04	5.05±0.05^{b}	5.21±0.07	5.77±0.02	ns
60	5.21±0.06	6.20±0.09^{ab}	5.75±0.02	6.05±0.07	ns
90	4.39±0.07^{B}	7.07±0.13^{Aa}	6.11±0.11^{A}	6.01±0.08^{A}	**
Sig.	ns	**	ns	ns	

$^{A-C}$ Mean values in the same row (different treatment in same day) with different cap ital letters indicate significant difference; a-c Mean values in the same column (same treatment in different days) with different lowercase letters indicate significant difference. Sig.: significance; n.s.: not significant; * P<0.05; ** P<0.01; *** P<0.001.

3.7. Sensory analysis

Regarding the sensory evaluation of cooked product (Fig. 2a), all the batches obtained an acceptance test score lower than three (acceptability limit), and there was no difference (P>0.05) in the odour of the sausages. However, CHIA treatment had the least acceptance of taste (2.82), whereas the LINS batch showed no difference compared to CONT group, indicating that the addition of linseed oil did not change the acceptability of the cooked lamb sausage. This outcome could be related to the formation of undesirable volatile compounds during cooking, whereas this difference was not detected in the raw product. In addition, the higher PUFA content observed in the CHIA group could favor the oxidation of the product, since the lipid oxidation rises with the unsaturated groups (double bonds) (Carvalho et al., 2019). Several authors have also reported sensory impairment by replacing animal fat with chia oil (Heck et al., 2017; Heck et al., 2019). This was reflected in the preference ordination of the different treatments, since less than 20% of consumers would choose CHIA as the most preferred. In contrast, CON obtained the highest scores, being chosen by more than 50% of panelist (Fig. 2b).

Figure 2. Sensory scores assigned by panelists (a) and preference (b) attributed by the panelist on day 0 on cooked lamb sausages produced with backfat, chia, linseed, or olive oils (■ CON, ■ CHIA, ■ LINS, ■ OLIV). Hedonic scale used: 1=excellent; 2=good; 3=acceptable; 4=hardly acceptable; 5=not acceptable. Error bars corresponding to standard error.

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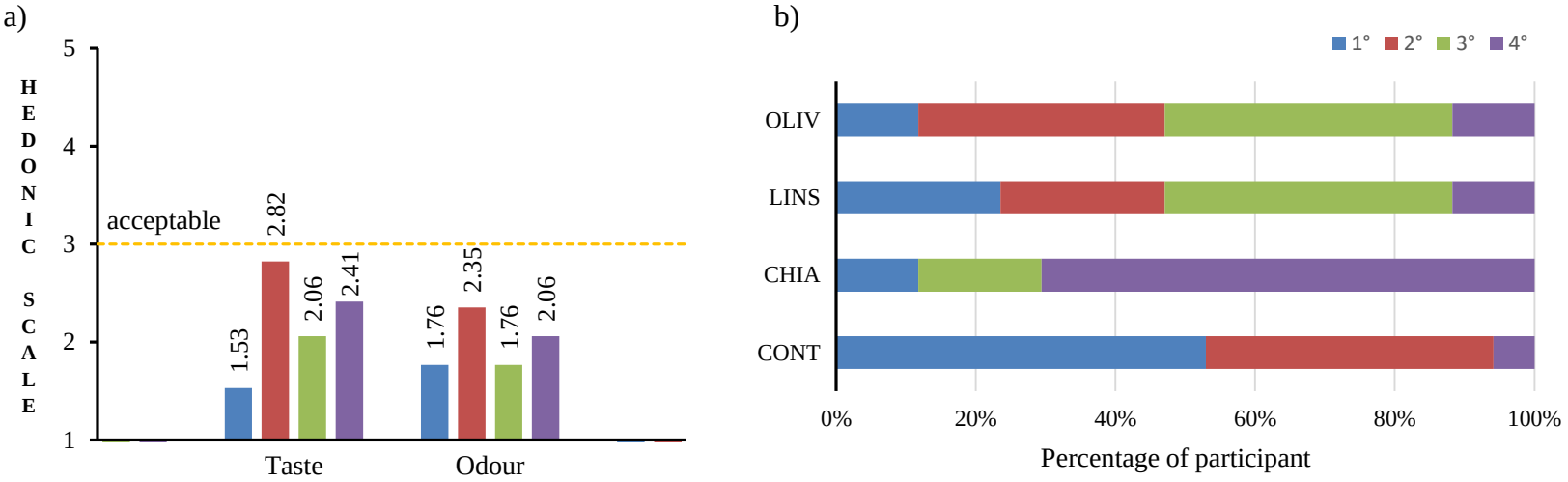
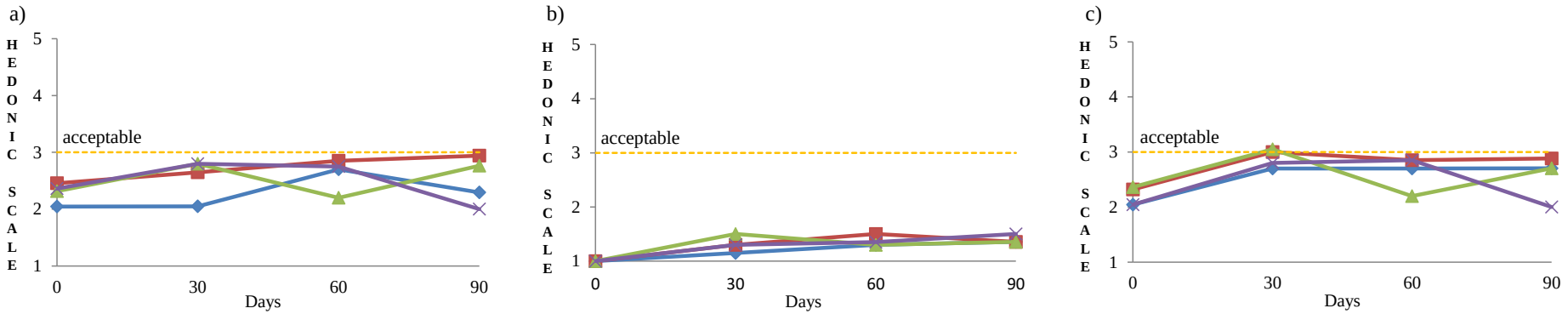


Concerning the sensorial analysis undertaken on the raw product (Fig. 3), throughout its shelf life, there was no significant difference in color, discoloration, and odor ($P>0.05$) among batches and during the storage time showing acceptable levels in all batches/time. Although the score values increased along the shelf-life of cooked lamb sausages, the values were below the score considered as the limit of acceptability that would mean the rejection of the product (Camo, Beltrán, & Roncalés, 2008). These results could be related to the lowest values obtained for TBARs values, in all cases lower than 0.6 mg MDA/kg which would be necessary to appreciate oxidized flavours by the consumer (Greene & Cumuze, 1982; Martínez, Cilla, Beltrán, & Roncalés, 2006).

Fig. 3. Sensory scores assigned by panelists on days 0, 30, 60, and 90 for color (a), discoloration (b), and odor (c) of cooked lamb sausages, produced with back fat, chia, linseed, or olive oils during storage (♦ CON, ■ CHIA, ▲ LINS, × OLIV). Hedonic scale used: 1 = excellent; 2 = good; 3 = acceptable; 4 = hardly acceptable; 5 = not acceptable.



4. CONCLUSION

Vegetable oils gave consistent improvements in the fatty acid profile and nutritional indexes of the cooked lamb sausages; although not so marked in the OLIV group. Regarding the physico-chemical parameters of the products, sausages elaborated with chia and olive oils showed a loss in texture parameters, which was noticed in the sensory analysis. On the other hand, LINS was able to enhance the lipid profile without alter its technological and sensory characteristics. For these reasons, among the oils tested, linseed oil is most recommended as a fat replacer in cooked lamb sausages.

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CHAPTER III - Turmeric (*Curcuma longa* L.) extract on oxidative stability, physicochemical and sensory properties of fresh lamb sausage with fat replacement by tiger nut (*Cyperus esculentus* L.) oil

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Abstract

This study aimed to evaluate the effect of different levels of turmeric extract on the physicochemical and sensory characteristics and oxidative stability of modified atmosphere-packaged fresh lamb sausages with fat replacement during storage (2 °C). Five treatments were prepared: control without antioxidant (CONT); with 500 mg/kg sodium erythorbate (E500); and three batches with 250, 500 or 750 mg/kg turmeric extract (T250, T500 and T750), respectively. The sausages were analyzed for their proximate composition (moisture, protein, fat and ash) and sensory acceptance (day 0); pH, color (L*, a*, b*), texture profile analysis (TPA), oxidative stability and visual sensory evaluation (0, 6, 12 and 18 days); free fatty acids and volatile compounds (0 and 18 days). Turmeric extract showed high antioxidant capacity and was able to retard lipid oxidation and generation of volatile compounds from oxidation in fresh lamb sausages. Thiobarbituric acid reactive substances (TBARs) values in natural antioxidant added samples were lower than control and sodium erythorbate ones, without compromising the physical chemical parameters. All samples were considered acceptable by consumers. These findings show that turmeric extract is effective against lipid oxidation and may be a strategy for extending the shelf life of meat products.

Keywords*:* antioxidant activity; *curcuma longa* L.; fat replacement; natural antioxidant

1. INTRODUCTION

The quality of meat and meat products is influenced by many factors throughout their shelf life, such as temperature, presence of oxygen and light, and microbial activity (de Carvalho et al., 2019). Consequently, the color, taste, nutritional value and other aspects of meat quality are changed, influencing the acceptance of these products (Chauhan, Das, Nanda, Kumbhar, & Yadav, 2018; Lorenzo et al., 2014).

Oxidation (lipid and protein) is one of the main causes of meat deterioration and may cause other undesirable effects, such as loss of essential fatty acids and amino acids, texture, taste, and discoloration (Lund, Heinonen, Baron, & Estévez, 2011; Zamuz et al., 2018).

Additionally, there is a relevant concern about fat levels in meat and meat products (MONTEIRO et al., 2017), and a growing demand for low-fat (DA SILVA et al., 2019) and/or with improved lipid profile products. Lipid reformulation by total or partial replacement of animal fat with unsaturated fat substitutes can provide healthier characteristics to the food product, meeting the demands of health-conscious consumers (Agregán et al., 2018; Carvalho et al., 2019; Lorenzo, Munekata, Pateiro, Campagnol, & Domínguez, 2016).

Tiger nut oil has a high amount of unsaturated fatty acids (HU et al., 2018) and can replace animal fat in meat products (BOBRENEVA; BAIOUMY, 2018). However, oils with higher contents of unsaturated fatty acids are more rapidly oxidized, leading to rancidity. Synthetic antioxidants are often used during processing to prevent or delay oxidation reactions. Alternatively, through a demand for natural and healthy products, several studies have evaluated the use of plant extracts as antioxidants in meat and lamb meat products (de Carvalho et al., 2019; Fernandes et al., 2017; Lorenzo et al., 2018; Pateiro et al., 2018).

Curcuma longa L. (turmeric) is an herbaceous plant widely used in Asian countries for its flavor, color and preservative properties. It has been widely studied for its high antioxidant capacity (MANCINI et al., 2015). Thus, this paper evaluated the effect of different levels of turmeric extract, as natural antioxidant, on the physical–chemical and sensory characteristics, as well as protein and lipid oxidation of modified atmosphere-packaged fresh lamb sausages with animal fat substitution with tiger nut oil during storage at 2 °C.

2. MATERIAL AND METHODS

2.1. Plant material and extract preparation

Commercial turmeric roots (purchased in the state of São Paulo, Brazil) were dried in a forced-air oven (Marconi, MA 035, Brazil) at 40 °C for 24 h (until constant weight), and then ground in a knife mill (Marconi, MA 340, Brazil). The extraction was carried out at 300 bar pressure and 70 °C using the TharSFC (Waters, Milford, MA, USA) system. The fixed-bed packing was composed of 80 g of dried and made to 290 mL (volume of reactor) with 3 mm diameter glass beads in each extraction. The static time (contact time between supercritical CO_2 and plant matrix before dynamic extraction) was set at 20 min and the dynamic extraction time set at 5 hours with a CO_2 flow rate of 10 g/min. Ethanol was used as a co-solvent (flow rate of 15 g/min) in order to improve the extraction as described by Bittencourt et al. (2019).

2.2. Determination of antioxidant capacity of radish and beetroot powders

2.2.1. Determination of Total Phenolic Content (TPC)

Total phenolic content was determined as described by Singleton, Orthofer, and Lamuela-Raventós (1999), with some modifications. The total phenolic content was determined using the Folin-Ciocalteu Reagent, with gallic acid as a standard. Appropriate dilutions of radishes and beetroots powders were prepared with water in test tubes (0.5 mL) and added of 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with water) and 2 mL of Na_2CO_3 solution (7.5%). The reaction occurred at 50 °C for 15 min, and absorbance was measured at 760 nm. The total phenolic content was expressed as mg of gallic acid equivalent (GAE)/100 g sample.

2.2.2. DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The DPPH scavenging method was performed with some modifications according to the procedure previously described by Brand-Williams, Cuvelier, and Berset (1995). 100 µL of samples were added to 3900 µL of DPPH solution (60 µM in methanol). After an incubation at 37 ºC for 10 min, absorbance readings were

performed at 515 nm. The DPPH scavenging activity of extracts was determined using Trolox as standard and the results were expressed as mg Trolox/g sample.

2.2.3. ABTS Radical Cation Decolourization Assay (ABTS)

ABTS radical cation decolourization assay was determined using the method described by Re et al. (1999), with some modifications. This assay consists on measuring the capacity to scavenge the ABTS radical (2,2-azinobis-(3-ethyl-benzothiazoline-6-sulphonate), which consist in measuring the reduction in the absorbance of the solution at 734 nm (from the characteristic ABTS radical blue-green colour to a colourless solution). The ABTS radical solution was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate and leaving the mixture in darkness at room temperature for 12-16 h before its use. In the following step, ABTS solution was diluted with PBS (pH 7.4) to get an absorbance of 0.70 at 734 nm, being equilibrated at 30 ºC. The working solution of ABTS (980 µL) was added to an aliquot of each sample (20 µL). The absorbance was measured at 734 nm after 10 min in darkness. The radical scavenging capacity was determined using the standard curve of ascorbic acid and the results were expressed as mg of ascorbic acid/100g sample.

2.2.4. Ferric Reducing Antioxidant Power (FRAP)

Ferric reducing antioxidant power was determined using the method described by Benzie and Strain (1996), with some modifications. The FRAP reagent was freshly prepared from acetate buffer 0.3 M (pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ) 10 mM in 40 mM HCl, and $FeCl_3$:$6H_2O$ 20 mM solutions. The three solutions were mixed together in the ratio 10:1:1 (v:v:v). Then, 900 µL of freshly-prepared FRAP reagent were mixed with 30 µL of properly diluted samples and 90 µL of distilled water. The mixture was heated at 37 ºC and left at this temperature during the reaction. After 20 min, the absorbance was measured at 593 nm. The FRAP value was expressed as µmol $Fe+^2$/100g sample based on a calibration curve prepared using $FeSO_4$ as standard.

2.2.5. Oxygen Radical Absorbance Capacity Assay (ORAC)

The ORAC assay was assayed according to the protocol of Huan, Ou, Hampsch-Woodill, Flanagan, & Prior (2002), with the following modifications. The reaction was carried out in 75 mM phosphate buffer (pH 7.4), with a final reaction

mixture volume of 200 µL. Then, 25 µL of dilute sample and 150 µL of 0.8 µM fluorescein (oxidizable substrate) were added into the internal wells of a black 96-well microplate (FluoroNunc™ F96-MicroWell™ plate) and immediately incubated at 37 °C for 30 minutes in the fluorescence instrument. Then, 25 µL of AAPH 184 mM (2,2-azobis(2-methylpropionamidine)dihydrochloride) solution was added rapidly to each well, using the fluorescence device injectors to initiate the reaction in the microplate reader. The fluorescence was recorded with excitation and emission filters of 485 and 528 nm, respectively. Samples were stirred prior to each reading. Trolox reagent was used as a standard reference compound and phosphate buffer was used as blank. Results were calculated on the basis of the differences of areas under the curves of fluorescence decay of the fluorescein between the blank and the sample (net area under the curve). They were expressed as mg Trolox/g sample.

2.3. Manufacture of fresh lamb sausages

The fresh lamb sausages are traditionally formulated with about 20% of animal fat. However, with the aim to produce a healthy product, they were elaborated with total replacement of animal fat with tiger nut oil emulsified in Prosella (gelling agent) and antioxidant turmeric extract at the Galicia Meat Technology Center (San Cibrao das Viñas, Spain). Five treatments were prepared: control (without antioxidant), E500 (500 ppm sodium erythorbate), C250 (250 ppm turmeric extract), C500 (500 ppm turmeric extract), and C750 (750 ppm turmeric extract). The ingredients used were 3.42 kg lamb meat; 0.9 kg fat emulsion, 0.06 kg salt and 0.09 kg water. For the tiger nut emulsion preparation, a mixture of tiger nut oil, Prosella powder [composed of jellifying agents (calcium sulphate and sodium alginate), wheat glucose syrup (7.4%), a stabilizer (disodium diphosphate, added P2O5: 9.58%) and an antioxidant (sodium ascorbate)] (Prosella VG NF4, Colin Ingredients, Mittelhausen, France) and water was elaborated as follow: water (56 g/100 g) and tiger nut oil (37.3 g/100 g) were mixed for 1 min in a bowl cutter (Sirman, mod C15VV, Marsango, Italy) at 3,000 rpm. The Prosella powder (6.7 g/100 g) was added and homogenized during 3 min and then left rest for 2 h. Finally, the mixture was refrigerated at 4 °C until needed. The tiger nut oil was characterized by high content of oleic acid (67-69%), followed by palmitic acid (14%), linoleic acid (10%) and stearic acid (3.5-5%) (Roselló-Soto et al., 2019; Vargas-Ramella et al., 2020). The choice of this oil was based on previous studies taking into account the nutritional benefits of meat products reformulated with partial or total

replacement of animal fat by this oil and that tiger nut oil does not affect or even improve the sensory quality and the acceptability of the meat products formulated with this oil (Barros et al., 2020; Vargas-Ramella et al., 2020).

The sausages were stuffed in collagen casings (diameter 30 mm) with an automatic stuffing machine (Junior, Sia, Barcelona, Spain) coupled to a vacuum packing equipment (VAE-10, Andher, Ciudad Real, Spain). After manufacture, sausages were packed under modified atmosphere (80% O_2 and 20% CO_2) in 300 mm thick PET-EVOH-PE trays, sealed with multilayer PE-EVOH-PE film (74 mm thick, permeability <2 mL/m^2 bar/day (Viduca, Alicante, Spain)) using a heat sealer (LARI3/Pn T-VG-R-SKIN, Ca.Ve.Co., Palazzolo, Italy). The samples were stored at 2 ± 1 °C under light, simulating the conditions from the supermarket and analyzed at 0, 6, 12 and 18 days of storage (proximate composition on day 0; pH, color parameters, oxidative stability and texture profile analysis on days 0, 6, 12 and 18; and free fatty acids and volatile compounds on days 0 and 18).

The whole experiment was repeated with the same ingredients and formulation in two batches separated two months in time, totaling 200 samples (5 treatments x 4 time periods x 5 repetitions x 2 runs).

2.4. Proximate composition

Protein, moisture and ash contents were determined according to International Standards Organization procedures (ISO 937, 1978; ISO 1442, 1997 and ISO 936, 1998, respectively). While the approved procedure Am 5-04, recognized by the American Oil Chemists' Society (AOCS, 2005), was used to quantify the total fat.

2.5. pH and color parameters

For pH measurement of sausages, a penetration pH meter was used (Hanna Instruments, Eibar, Spain). After the sausages had been exposed to the atmosphere for 10 minutes, color parameters were measured in the CIELAB space (lightness, *L**; redness (+)/greenness (-), *a**; yellowness (+)/blueness (-), *b**) using a portable colorimeter (CR-600d, Minolta Co. Ltd., Osaka, Japan).

2.6. Cooking loss and texture profile analysis test

The sausages were cooked in a water bath up to their geometric center achieve 70 °C. The temperature was monitored with the K-type thermocouples (Comark, PK23M, UK) connected to a data logger (Comark Dilligence EVG, N3014, UK). Cooking loss was then measured by the weight difference between cooked and raw samples.

Texture profile analysis (TPA) (hardness, springiness, cohesiveness and chewiness) was measured in sausage slices of 2 cm by compressing to 50% (cylindric probe with flat surface area of 19.85 cm^2) and the force-time curves were recorded at 1 mm/s crosshead speed. Texture parameters were obtained using a texture analyser TA.XTPlus (Stable Micro Systems, Vienna Court, UK).

2.7. Antioxidantp activity and oxidative stability (lipid and protein)

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydraz (DPPH) method, in which one gram of lyophilized sample was homogenized with 20 mL of methanol using an IKA T25 Ultra-Turrax digital (IKA®-Werke GmbH & Co. KG, Staufen, Germany), centrifuged at 10,000xg/10 min and filtered. The antioxidant activity was calculated from the standard curve obtained from a Trolox solution (0.03 g Trolox in 100 mL methanol) prepared under darkness, which was diluted into seven different concentrations ranging from 0 to 1.2 mM Trolox to construct the standard curve. A 3.9-µL aliquot of 60 µM methanolic DPPH solution was added to 100 µL of the sample and Trolox solutions (T1–T7), homogenized in a vortex and incubated at 37 °C/10 min.

The percentage of DPPH radical inhibition was calculated by the difference between the blank and sample measurements at 515 nm ((blank absorbance − sample absorbance / blank absorbance) x 100). Blank was considered as the DPPH absorbance value measured at time 0 (0.1 mL methanol in 3.9 mL DPPH). The antioxidant capacity of the samples was calculated by replacing the percentage inhibition on the Trolox standard curve, which was expressed in µg Trolox/g of sample.

Lipid oxidation was evaluated by measuring the TBARs index (development of the thiobarbituric acid reactive substances, secondary products of the lipid oxidation) according to Vyncke (1975), the results were expressed as milligrams of malonaldehyde (MDA) per kilogram of sample. Protein oxidation was assessed by carbonyl formation (product of the protein oxidation) according to Mercier, Gatellier, and Renerre (2004), and expressed as nmol of carbonyl/mg of protein.

2.8. Volatile compounds

Solid-phase microextraction was performed using fused-silica fiber (10 mm length) coated with a 50/30 µm thickness of DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane). To determine the volatiles, conditioning, extraction (37 °C for 30 min after an initial temperature equilibrium at the same temperature for 15 min) and injection of the samples were performed with a PAL-RTC 120 autosampler. The fiber was transferred to the injection port of a gas chromatograph (7890B Agilent Technologies) with a 5977B selective mass detector (Agilent Technologies) and a DB-624 capillary column (30 m ± 0.25 mm id, film thickness). 1.4 µm; J&W Scientific, Folsom, CA, USA).

To identify volatile compounds, their mass spectra were compared with those contained in the NIST14 library and/or by comparing their mass spectra and retention time with authentic standards (Supelco, Bellefonte, PA, USA); and/or by the retention index relative to a series of standard alkanes (C5−C14) (to calculate the linear retention index; Supelco 44585-U), and associate them with the data defined in the literature. Results were expressed in units of area (AU) × 10^4/g of sample.

2.9. Sensory analysis

To determine whether or not the participant liked the lamb sausage with added turmeric extract, an acceptance test was conducted in individual standardized booths at Galicia's Meat Technology Center. Forty panelists assessed the attributes of color, surface discoloration and odor in raw sausages during their shelf life using a 5-point hedonic scale (1 = not acceptable; 5 = excellent); while in cooked sausages (70 °C), the attributes of color, flavor, texture and overall quality of cooked sausages were

evaluated using a 7-point hedonic scale (from 1 = dislike very much to 7 = like very much, very much liked). Samples were coded with random three-digit numbers and presented to panelists on plastic plates along with water and unsalted toasted bread to clean the palate and remove residual flavors.

2.10. Statistical analysis

Statistical analyses were performed using SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Normal distribution and variance homogeneity had been previously tested (Shapiro–Wilk). The data were submitted to two-way analysis of variance (ANOVA)(treatment and storage time as a fixed effect, and replicate as a random effect), or a one-way ANOVA (for the variables assessed only at day 0), followed by Tukey's test when the ANOVA was significant ($p<0.05$). For sensory data, Friedman's analysis of variance by ranks was applied followed by the Wilcoxon test with Bonferroni correction for multiple pairwise comparisons when a significant difference in the Friedman's test was detected ($p<0.05$). Panelists were included in the model as a random effect.

3. RESULTS AND DISCUSSION

3.1. Total phenolic content and antioxidant capacity of turmeric extract

Several studies reported that phenolic compounds are the main contributors to antioxidant activity (Lorenzo & Munekata, 2016; Mirian Pateiro, Vargas, et al., 2018; Şahin et al., 2018; Shan, Cai, Sun, & Corke, 2005). The TPC contents obtained in the present study were higher than those obtained in other turmeric extracts (5018.42 vs. 67.90 mg GAE/100g, respectively) by other authors (Maizura, Aminah, & Wan Aida, 2011). This could be due to differences in the extraction methods used (Braga, Leal, Carvalho, & Meireles, 2003). Therefore, we can confirm that the extraction technique had influence on the quality and composition of the resulted extract, increasing the extraction of phenolic compounds among others (Mirian Pateiro, Barba, et al., 2018; Putnik et al., 2017; Putnik et al., 2018; Žlabur et al., 2020). The values found for turmeric were lower than the values found in other natural antioxidants as oregano and

rosemary extracts (517.21 and 115 mg GAE/g) (Fernandes, Trindade, Lorenzo, & de Melo, 2018; Pires et al., 2017).

The extraction method used, supercritical fluid extraction, is an emerging technology applied in the extraction of valuable compounds from natural sources (Koubaa et al., 2015). It has several advantages, since it is considered a green technology and a sustainable processing, avoiding the use of toxic organic solvents and reducing energy use and environmental pollution (Al Khawli et al., 2019).

Regarding antioxidant activity, several methods were used in order to understand which types of mechanisms are involved in the activity of the natural extracts (Huang, Boxin, & Prior, 2005). Among the diverse methods employed to determine the antioxidant capacity, those used in the present study are the most used. Turmeric extract displayed a high antioxidant capacity with values of DPPH and ABTS assays of 42.92 mg Trolox/g and 1490.53 mg ascorbic acid/100 g, respectively. FRAP assay displayed values of 980.27 µmol $Fe+^{2}$/100 g. These values were similar to those found by Jang et al. (2007) who noticed FRAP values of 972 μmol $Fe+^{2}$/100 g for turmeric extracts obtained with methanol and conventional extraction. In addition to the previous methods, which are commonly used to assess antioxidant capacity, turmeric extract was also evaluated using ORAC. This method is widely applied to evaluate the antioxidant activity of phenolic compounds. The results obtained displayed values of 71.72 mg Trolox/g, which were higher than those observed by other authors in aqueous and ethanol extracts ranging from 10 to 25 µmol Trolox/g (Tilak, Banerjee, Mohan, & Devasagayam, 2004).

3.2. Proximate composition

The effect of turmeric extract concentration on the proximate composition of sausages with total fat replacement with tiger nut oil is shown in Table 1. Not surprisingly, the chemical composition did not differ significantly between treatments ($P>0.05$) and had values in the ranges of 69.45−70.54% for moisture, 8.47−9.49% for fat, 15.16−15.92% for protein and 2.87−2.91% for ash. These results are consistent with the formulations used in this study and agreed with data reported in scientific literature for lamb sausage elaborated with natural antioxidants (Fernandes, Trindade, Lorenzo, Munekata, & de Melo, 2016).

3.3. pH and color parameters

The pH values were affected by storage time ($P<0.001$). The pH increased on day 6 for all treatments and then remain practically constant until the end of the storage (Table 1). Among treatments, there were significant differences at the beginning and at the end of the display period. The sausages with antioxidants had higher values than those observed in the control, with values with values of 5.57 or higher in samples with antioxidants (E500 and samples with turmeric) compared to the lower values in control samples. A similar outcome was observed on day 18, again control samples presented lower values than the treatments (5.51 vs. 5.57, 5.58, 5.61 and 5.63 for CONT vs. E500, C250, C500 and C750, respectively). The values obtained in this study were in agreement with data reported by other authors (de Carvalho et al., 2019; Lorenzo et al., 2018), who evaluated the influence of natural extracts in the shelf life of meat products. Moreover, these findings could be related to the proteolytic processes that occur during the shelf life. According to Carvalho et al. (2019), pH values can be affected by the degradation of proteins and liberation of peptides, amino acids, ammonia and amines during storage.

Regarding the color parameters, storage time did not affect L* values ($P>0.05$). Particularly for days 12 and 18, the control treatment presented the highest values (50.96 and 46.46, respectively). In the case of a* values, there was a significant decrease ($P<0.001$) throughout storage for all treatments, except for C500 sausages. Samples containing turmeric extracts showed the lowest a* values at days 0 and 18 (values in the ranges of 7.02-9.11 and 5.98-6.36, respectively). Despite presenting the lowest value of a*, the fact that there was no change in a* values of C500 sausages during storage ($P>0.05$) indicated greater stability and may represent an advantage over other treatments, since change in product color is not desired by consumers. Decreasing a* values of meat products added of natural antioxidants during storage have also been reported by other authors (Alirezalu et al., 2019; Mirian Pateiro, Vargas, et al., 2018; Zamuz et al., 2018).

Table 1. Effect of turmeric extract on pH and color parameters of fresh lamb fresh sausages with replacement of animal fat by tiger nut oil during cold storage (2 °C)

		Treatment						
	Day	CONT (n=40)	E500 (n=40)	C250 (n=40)	C500 (n=40)	C750 (n=40)	SE	Sig.
pH*	0	5.53Bb	5.57ABb	5.57ABb	5.57ABb	5.59Ab	0.01	*
	6	5.73a	5.76a	5.75a	5.72a	5.73a	0.01	ns
	12	5.55b	5.58b	5.58b	5.53b	5.55b	0.02	ns
	18	5.51Bb	5.57Ab	5.58Ab	5.61Aab	5.63Aab	0.01	***
	SE	0.01	0.01	0.01	0.02	0.01	-	-
	Sig.	***	***	***	***	***	-	-
L*	0	50.78	50.23	48.38	49.40	48.38	0.40	ns
	6	50.70	50.44	49.64	47.49	47.85	0.37	ns
	12	53.24A	51.06AB	50.93AB	49.18B	48.97B	0.40	*
	18	50.96A	50.90A	50.36AB	48.18BC	46.46C	0.29	***
	SE	0.41	0.42	0.39	0.39	0.42	-	-
	Sig.	ns	ns	ns	ns	ns	-	-
a*	0	10.44ABa	11.44Aa	9.11BCa	7.02D	8.16Ca	0.20	***
	6	7.87ABb	8.92Ab	6.98Bb	7.62AB	8.25ABa	0.16	**
	12	7.73Ab	7.01ABc	5.48Bb	6.27AB	7.55Aab	0.18	**
	18	7.94Ab	8.13Abc	6.18Bb	6.36B	5.98Bb	0.13	***
	SE	0.14	0.15	0.21	0.20	0.23	-	-
	Sig.	***	***	***	ns	***	-	-
b*	0	17.56C	18.54Ca	26.32B	31.87A	35.32A	0.42	***
	6	17.66C	18.36Ca	29.67B	32.56A	35.04A	0.34	***
	12	18.09C	16.54Cb	29.22B	32.46A	35.45A	0.35	***
	18	17.22D	17.38Dab	28.61C	31.62B	35.01A	0.32	***
	SE	0.20	0.20	0.55	0.47	0.45	-	-
	Sig.	ns	**	ns	ns	ns	-	-

A−D Different capital letters indicate a significant difference between treatments. a−c Different lowercase letters indicate a significant difference between storage times. SE: standard error; Sig.: significance; n.s.: not significant; ∗ p<.05. ∗∗ p<.01 ∗∗∗ p<.001.

In the case of b* values, significant differences (P<0.05) were observed only in E500 sausages during storage. It is relevant mentioning that, as expected, there was a gradual increase in b* value with the addition of turmeric extract (P<0.05). The C750 batch had the highest values at the beginning and the end of shelf life (35.32 and 35.01, respectively). These results can be attributed to the characteristic yellow color of turmeric. In the food industry, turmeric powder is commonly used as a yellow colorant. A similar outcome was reported by Mancini et al. (2015) who noticed higher a* values in rabbit burger containing turmeric powder than in control treatment.

3.4. Cooking loss and texture profile analysis test

Regarding cooking losses, on day 0 there was a variation from 21.16% to 26.67% among groups (P<0.05), when C250 sausage presented less cooking loss than the control treatment (Table 2). Only sausages containing turmeric extract were affected by storage time (P<0.05), wherein an increasing until day 18 was observed. The sausages elaborated with turmeric extract reached values similar to the control (25.08%) but higher than the E500 batch (21.78%) at the end of storage period. Das, Rajkumar, and Dwivedi (2011) also reported an increase of cooking losses over the storage period by adding curry leaf (Murraya koenigii) powder in cooked ground goat meat. According to Kumar, Kairam, Ahmad, and Yadav (2016), cooking loss can be influenced by the ability of the protein matrix to stabilize and/or immobilize fat and water molecules. Additionally, phenolic compounds may interact with the protein thiols to modify water retention capacity, especially at high concentrations (Pateiro, Bermúdez, Lorenzo, & Franco, 2015).

The addition of antioxidants did not affect the texture parameters (P>0.05) during storage period. On the other hand, hardness, springiness and chewiness increased over time (P<0.01). The hardness of the sausages had minimum value of 33.89 N on day 0 and maximum of 64.62 N on day 18, while for springiness and chewiness the minimum values were 0.75 mm and 13.12 N.mm and maximums were 0.82 mm and 28.97 N.mm, respectively. These results can be explained by the possible loss of moisture during the storage time. Pateiro et al. (2015a) noticed

significant correlations of hardness, chewiness and cohesiveness with moisture content ($r = -0.67$, $r = -0.48$, $r = -0.26$, respectively). In addition, according to Estévez, Ventanas, and Cava (2005), protein oxidation can cause an increase in hardness due to loss of protein functionality and the formation of protein crosslinks. These authors reported a significant correlation between carbonyl content and hardness ($r = 0.56$).

Table 2. Effect of turmeric extract on cooking loss and texture profile analysis of fresh lamb sausage during refrigerated storage.

		Treatment						
	Day	CONT (n=40)	E500 (n=40)	C250 (n=40)	C500 (n=40)	C750 (n=40)	SE	Sig.
Cooking loss (%)	0	26.67A	22.79AB	21.16Bb	23.85ABab	23.68ABb	0.49	*
	6	24.22A	20.45B	24.33Aab	22.92ABab	23.86ABab	0.40	*
	12	23.66	20.48	23.33ab	21.88b	23.04b	0.44	ns
	18	25.08AB	21.78B	25.95Aa	25.73Aa	26.94Aa	0.42	**
	SE	0.50	0.57	0.46	0.48	0.41	-	-
	Sig.	ns	ns	**	*	**	-	-
Hardness (N)	0	37.18c	39.60b	33.89b	39.50b	37.51b	1.04	ns
	6	53.14b	60.65a	52.46a	54.45a	52.98a	1.33	ns
	12	56.78b	65.70a	59.58a	63.34a	55.74a	1.60	ns
	18	64.62a	64.32a	56.24a	64.69a	51.90a	1.57	ns
	SE	0.93	1.83	1.87	1.48	1.55	-	-
	Sig.	***	***	***	***	***	-	-
Springiness (mm)	0	0.75b	0.77b	0.76b	0.79b	0.78b	<0.01	ns
	6	0.80a	0.82a	0.80a	0.81ab	0.82a	<0.01	ns
	12	0.80a	0.83a	0.83a	0.83a	0.82a	<0.01	ns

	18	0.80a	0.82a	0.82a	0.82a	0.81ab	<0.01	ns
	SE	<0.01	<0.01	0.01	<0.01	0.01	-	-
	Sig.	***	***	***	***	*	-	-
Cohesiveness	0	0.51	0.51	0.51	0.52	0.51	<0.01	ns
	6	0.55	0.51	0.53	0.53	0.53	0.01	ns
	12	0.55	0.54	0.54	0.53	0.53	0.01	ns
	18	0.54	0.55	0.52	0.53	0.51	0.01	ns
	SE	0.01	0.01	0.01	0.01	0.01	-	-
	Sig.	ns	ns	ns	ns	ns	-	-
Chewiness (N.mm)	0	14.19c	15.54b	13.12b	16.19b	14.86b	0.52	ns
	6	23.00b	25.95a	22.35a	23.39a	22.89a	0.76	ns
	12	24.74ab	29.79a	26.88a	27.82a	24.23a	0.90	ns
	18	27.91a	28.97a	24.00a	27.90a	21.67ab	0.90	ns
	SE	0.42	1.00	1.04	0.84	0.93	-	-
	Sig.	***	***	***	***	**	-	-

A−C Different capital letters indicate a significant difference between treatments; a−c different lowercase letters indicate a significant difference in the same treatment overtime. SE: standard error; Sig.: significance; n.s.: not significant; $*$ $p<0.05$. $**$ $p<0.01$ $***$ $p<0.001$

3.5. Antioxidant activity and toxidative stability (lipid and protein)

The changes in the antioxidant activities of lamb sausages assessed by DPPH method are shown in Fig. 1A. At the beginning of storage, samples with antioxidants (either natural or synthetic) presented higher DPPH value compared to control. Unsurprisingly, DPPH values decreased over time ($P<0.001$) in all batches up to day 6 when the antioxidant activity of sausages was steady until the end of storage. During the whole display, the C750 batch presented the highest values, changing from 995.18 to 418.37 μg Trolox/g at the beginning and end of storage time. It is important to highlight that C750 treatment displayed higher values than E500 batch throughout the storage period. A similar behavior was observed in the other turmeric treatments although only in some sampling points. In this way, C500 treatment showed more antioxidant DPPH activity than E500 batch from day 6 of sampling to the end of storage period, while C250 treatment only had higher values in the last two sampling points of the shelf life. This outcome supports the effectiveness of turmeric extract to improve and sustain the antioxidant potential of lamb sausages during storage.

Several studies have evaluated the effect of incorporating natural antioxidants into meat products (Cunha et al., 2018; Domínguez et al., 2020; Echegaray et al., 2018; Fernandes et al., 2017; Lorenzo, González-Rodríguez, Sánchez, Amado, & Franco, 2013; Lorenzo et al., 2018; Pateiro, Bermúdez, et al., 2015; Pateiro, Lorenzo, Vázquez, & Franco, 2015). However, in general, a higher dose than synthetic antioxidant is required. In the present study, at a lower dose than that of sodium erythorbate, we observed high antioxidant capacity in sausages elaborated with turmeric extract and the maintenance of high levels over time compared to treatments synthetic antioxidant.

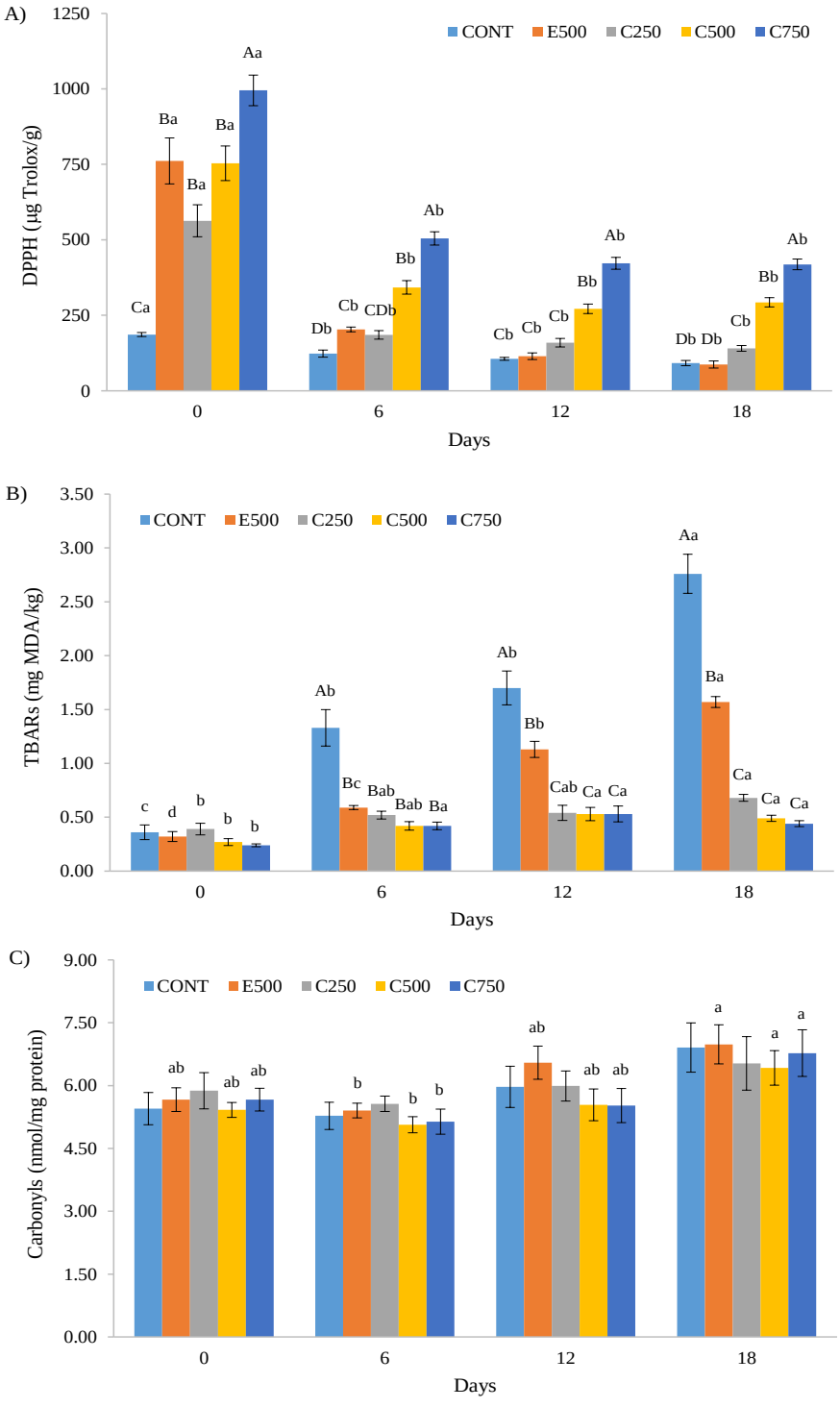
The main antioxidant compounds of turmeric are curcuminoids, among which curcumin is the most widely studied (Mancini et al., 2015). According to Das and Das (2002), curcumin is a potent singlet oxygen-quencher and can be regenerated by secondary antioxidants. It is positioned within the cell membrane, where it intercepts lipid radicals and becomes a phenoxyl radical. Moreover, this radical is more polar than curcumin and can move towards the membrane surface and be repaired by any water-soluble antioxidant such as ascorbic acid (Ak & Gülçin, 2008). Moreover, Jayaprakasha, Rao, and Sakariah (2006) reported that two other curcuminoids (demethoxycurcumin and bisdemetoxycurcumin) were also effective antioxidant compounds found in turmeric.

Lipid oxidation is one of the main factors limiting the shelf life of meat products (Domínguez, Pateiro, et al., 2019). The evolution of TBARS values of lamb sausages with turmeric extract over time is shown in Fig. 1B. The antioxidant addition and storage time influenced MDA content ($P<0.01$). As expected, on day 0 non-significant differences among batches were observed. TBARS levels increased over time in CONT (from 0.36 to 2.76 mg MDA/kg on days 0 and 18, respectively) and E500 (from 0.32 to 1.57 mg MDA/kg on days 0 and 18, respectively) batches, while samples with turmeric exhibited an irregular behavior. In this regard, C500 and C750 treatments increased until day 12 and then decreased slightly until the end of storage time. Samples with the low dose of turmeric showed a stabilization stage between days 6 and 12, and then continued to increase. The values of these samples were lower than those observed in control and E500 treatments, but slightly higher than the samples with higher turmeric contents. It is relevant mentioning that this behavior is inversely proportional to the DPPH value of the respective treatments, supporting the effectiveness of its antioxidant power.

On day 6, treatments with added antioxidant showed lower levels of MDA compared to the control, but non-significant differences were observed among them. On days 12 and 18, control batch had the highest MDA levels (1.70 mg MDA/kg and 2.76 mg MDA/kg, respectively) followed by E500 treatment (1.13 mg MDA/kg and 1.57 mg MDA/kg, respectively), while sausages with turmeric extract showed better oxidative stability even when compared with the E500 group. Among the turmeric extract treatments, the highest TBARS value was observed in C250 treatment on day 18 (0.68 mg MDA/kg), which was lower than those obtained for control and E500 batches (2.76 mg MDA/kg and 1.57 mg MDA/kg, respectively) after 18 days of storage. It is interesting highlight that Verma and Sahoo (2000) reported MDA concentrations between 1 and 2 mg/kg as limit values for the sensory perception of rancidity. Thus, the control samples in the present study would be perceived as rancid on the sixth day of storage, the E500 treatment on the 12th day, while the samples containing turmeric extract did not exceed the limit values until the end of the storage time. C500 and C750 treatments even showed values below 0.6 mg MDA/kg, considered as a more restrictive level of deterioration of the rancid flavor in meat products (Georgantelis, Blekas, Katikou, Ambrosiadis, & Fletouris, 2007).

Protein carbonyls are among the main products of the protein oxidation, and the carbonyl radical is usually used the indicator to evaluate the progression of protein oxidation (Lund et al. 2011). The changes of total carbonyl content of lamb sausages during storage are presented in Fig. 1C. Carbonyl levels decreased ($P>0.05$) during the first 6 days and then their levels increased progressively until the end of storage time. This increase was significant in E500, C500 and C750 treatments, with results ranging from 5.66, 5.42 and 5.67 nmol/mg on day 0 to 6.98, 6.42 and 6.77 nmol/mg at 18 days, respectively. Our results agree with those reported in pork patties with guarana seed extract as natural antioxidant (Pateiro, Vargas, et al., 2018). These authors reported in samples treated with guarana extract a reduction of carbonyls content during the first 7 days and then, a progressive increase during the storage was observed. Thus, our results suggest that during initial stages occur a degradation of carbonyls in other oxidative compounds. This theory was probed by other authors in cooked lamb loin, in which detected the degradation of carbonyls due to reactions with other compounds which promoted carbonyls reduction (Roldan, Antequera, Armenteros, & Ruiz, 2014). In contrast, other authors reported an increase of carbonyls during the whole display. Agregán et al. (2019), who tested the effect of Fucus vesiculosus extracts as natural antioxidants in pork patties formulated with oleogels during 18 days of storage found a progressive and continuous increase of carbonyls content. Finally, other authors reported in sheep burgers (Fernandes et al., 2016), lamb burgers (Fernandes et al., 2017) and sheep sausages (Fernandes et al., 2018) different behavior in carbonyls content (increase or decrease) in function of the sampling point. Additionally, in the present study there was no significant difference ($P>0.05$) among treatments at any of the evaluated intervals. In contrast, other authors reported that the use of oregano extract in lamb burger (Fernandes et al., 2017), the use of guarana extract at low concentrations (Pateiro, Vargas, et al., 2018) or pitanga leaf extract (Lorenzo et al., 2018) in pork burgers resulted in a significant reduction of carbonyls formation during the storage time.

Figure 1. Evolution of antioxidant activity (DPPH) (A), TBARs values (B) and carbonyl content (C) in fresh lamb sausages (CONT − control; E500 − sodium erythorbate (500 ppm); C250, C500 and C750 − turmeric extract (250, 500 and 750 ppm, respectively). A−D Different capital letters indicate a significant difference between treatments. a−c Different lowercase letters indicate a significant difference between storage times. Error bars corresponding to standard error.



According to Lund et al. (2011), the mechanism of protein oxidation occurs through a free radical chain reaction comparable to lipid oxidation, but with greater pathway complexity and greater variety of oxidation products. In a complex matrix such as meat, the links between lipid peroxidation and protein oxidation are still unclear (Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015). In addition, Lund et al. (2011), argued that protein carbonylation can influence the functionality of proteins, including water retention capacity and texture. In this regard, Estévez, Ventanas, Heinonen, and Puolanne (2011) associated the intensity of protein carbonylation with a decreasing water-binding ability of the meat proteins. Although some authors indicated a correlation between protein and lipid oxidation, this relation was not observed in the present study. In this sense, this scenario suggests that carbonyl content was more related to texture parameters than lipid oxidation.

3.6. Analysis of volatile compounds

Volatile compounds formed during the lipid oxidation have unpleasant odors in meat products (Domínguez, Pateiro, et al., 2019). Aldehydes, ketones and alcohols are mainly produced from lipid oxidation reactions, and the generation of a disagreeable flavor is a critical issue that can affect the storage stability of meat products. Furthermore, volatile compounds analysis is a powerful tool to monitor changes in lipid oxidation processes (Domínguez, Pateiro, et al., 2019; Domínguez, Purriños, et al., 2019; Lorenzo, 2014). This technique allows us the determination of specific compounds derived from the hydroperoxide decomposition in more accurate way than other techniques. The lipid-oxidation derived volatile compounds from fresh lamb sausages, evaluated at the beginning and end of storage, are presented in Table 3. A total of 8 volatile compounds resulting from lipid oxidation were affected by shelf life: four aldehydes (hexanal, heptanal, octanal and 2-Octenal, (E)-) and four alcohols (1-butanol, 1-pentanol, 1-hexanol, 1-octen-3-ol). The samples treated with turmeric extract showed a completely different behavior in comparison with control or E500 treatmetns. In the aldehyde family, the contents of hexanal in samples treated with turmeric extract suffered a significant reduction from 13.34-19.94 AU x 10^4/g at the initial sampling point to 2.68-11.02 AU x 10^4/g at the end of storage time. In similar way, the content of octanal in the samples added with turmeric extract also showed a significant decrease from 1.3-1.6 AU x 10^4/g at day 0 to 0.21-0.59 AU x 10^4/g at day

18. In both cases, the addition of 500 ppm of turmeric extract (C500) was the concentration that presented the most antioxidant effect. In control and E500 samples, the content of hexanal ($P<0.01$) and octanal ($P>0.05$) was higher than in turmeric samples at initial and final sampling point. Furthermore, in contrast with the results observed for the C250, C500 or C750 samples, the content of these two aldehydes increased during the refrigerate storage in the case of control and E500 treatmetns, which demonstrated the lower antioxidant and protective effect of these two treatments in comparison with the turmeric extract.

On the other hand, the amounts of heptanal and 2-octenal (E) showed a significant increase during the refrigerate storage (except for C750 samples). However, similarly to the other aldehydes, the increment of heptanal in control (from 1.17 to 5.25 AU x 10^4/g) and E500 (from 1.25 to 6.67 AU x 10^4/g) treatments was significantly higher than in C250, C500 and C750 samples (from ~0.6 to 0.64-1.99 AU x 10^4/g). In the same way, although the content of 2-octenal (E) in the initial point did not show significant differences among batches (0.13-0.23 AU x 10^4/g), whereas at day 18 control and E500 samples had superior amounts of this aldehyde.

It is well known that hexanal has been considered to be the greatest indicator of lipid oxidation in meat products, since its content increases to a greater extent than that other aldehydes (Domínguez, Pateiro, et al., 2019). However, in more recent studies several researchers pointed that some of these volatiles are highly specific to the oxidation of particular fatty acids. In this regard, hexanal and 2-octenal deriving from the oxidation of n-6 fatty acids, mainly linoleic and arachidonic (Lorenzo & Carballo, 2015; Montanari et al., 2018), while heptanal and octanal derived from lipid oxidation of oleic acid (Domínguez, Pateiro, et al., 2019; Domínguez, Purriños, et al., 2019). Some unsaturated aldehydes as 2-octenal was proposed as lipid oxidation indicator; however saturated aldehydes are much more stables and thus more used.

In similar way to occurs in the present research, the addition of natural antioxidants was reported as an effective strategy to prevent the apparition of aldehydes in other meat products. A significant reduction was observed by Carvalho et al. (2019) who used guarana and pitanga extracts as natural antioxidants in lamb burger elaborated with emulsified chia oil. The additions of oregano extract to sheep burgers also reduce the contents of heptanal at day 0 and after 20 days of refrigerate storage (Fernandes et al., 2016). The same authors also found a significant effect of

oregano extract in hexanal and heptanal amounts of sheep cooked sausages. However, in this case, the dose effect of oregano extract promoted or inhibited the aldehydes formation (Fernandes et al., 2018). The addition of beer residue extract, chestnut leave extract and peanut skin extract to dry-cured sausage formulated with high long-chain n-3 PUFA also resulted in a significant reduction of hexanal content (Munekata, Domínguez, Franco, et al., 2017). In contrast, the use of this extracts (beer, chestnut and peanut) did not influence the amounts of heptanal in pâtés formulated with a mixture of olive and fish oil at day 0 or after 160 days of storage (Munekata, Domínguez, Campagnol, et al., 2017). The content of hexanal only was reduced with the chestnut leave extract at day 0, while at day 160 any extracts exerted effect on hexanal formation (Munekata, Domínguez, Campagnol, et al., 2017).

The reduction of hexanal amount found in the present study during the refrigerated period was also observed during the storage of liver pâtés formulated with high unsaturated fatty acids and beer, chestnut leave and peanut skin extracts as natural antioxidants (Munekata, Domínguez, Campagnol, et al., 2017). The antioxidant effect of extract inhibited the lipid oxidation and thus, the formation of hexanal. Additionally, the hexanal present in the samples could be reduced and produce 1-hexanol (Montanari et al., 2018), which agree with our results (Table 3). Therefore, the combination of these two effects resulted in a significant reduction of hexanal in samples with turmeric extract.

With regard to the alcohol family, the formation of 1-butanol and 1-pentanol were not affected by storage time in the treatments with turmeric extract. In the control group these compounds increased from 0.99 AU x 10^4/g to 2.58 AU x 10^4/g and from 39.80 AU x 10^4/g to 2284.73 AU x 10^4/g between days 0 and 180, respectively. In the E500 group, 1-butanol and 1-pentanol increased from 0.73 AU x 10^4/g to 1.83 AU x 10^4/g and from 37.86 AU x 10^4/g to 182.26 AU x 10^4/g between the first and last day of storage, respectively. On the other hand, 1-hexanol and 1-octen-3-ol increased over time in all groups, but to a lesser extent in treatments with turmeric extract. The C750 treatment had the lower values (42.79 AU x 10^4/g and 16.21 AU x 10^4/g, for 1-hexanol and 1-octen-3-ol, respectively) compared with control group (1490.60 AU x 10^4/g and 148.20 AU x 10^4/g, for 1-hexanol and 1-octen-3-ol, respectively). These compounds are derived mainly from oxidative degradation of fatty acids and are frequently reported in the scientific literature and considered as meaningful indicators of lipid oxidation in

meat (Maggiolino et al., 2019; Resconi et al., 2018). Contrary to our results, the use of oregano extract did not exert any effect on 1-pentanol content on sheep burgers at day 0 or after 20 days of storage time (Fernandes et al., 2016).

In fact, due to its low threshold, 1-octen-3-ol was described as important volatile contributing to the characteristic aroma of dry-cured meat products. This compound derives from the autoxidation of linoleic acid (Domínguez, Purriños, et al., 2019). Similarly, 1-pentanol and 1-butanol arise from the degradation of lipid hydroperoxides (Domínguez, Purriños, et al., 2019), while the 1-hexanol derived from the reduction of hexanal (Montanari et al., 2018). Thus, the increase of 1-hexanol content in the samples treated with turmeric extracts could be related with the degradation (reduction) and diminution of hexanal amounts found in these samples. Moreover, the very high amounts of both, hexanal and 1-hexanol in control and E500 samples could be due to a continuous formation and reduction of hexanal, which resulted in a high amounts of both compounds.

With all results in mind, it eases to conclude that turmeric extract had a high antioxidant activity. The low and stable volatile amounts in these samples confirm the protective effect of turmeric extract against lipid oxidation and agree with TBARS results.

Table 3. Effect of turmeric extract on volatile compounds of lamb sausages during refrigerated storage (0 and 18 days) expressed as area units (AU) × 10^4/g of sample.

	m/z	LRI	R	Day	Treatments					SE	Sig.
					CONT (n=20)	E500 (n=20)	C250 (n=20)	C500 (n=20)	C750 (n=20)		
Hexanal	56	865	ms, lri	0	31.58ab	43.79a	19.94b	13.34b	17.02b	2.57	**
				18	72.62a	85.43a	11.02b	2.68b	5.44b	7.90	**
				Sig.	ns	ns	*	***	**	-	-
Heptanal	70	974	ms, lri, s	0	1.17ab	1.25a	0.66bc	0.54c	0.56c	0.07	***
				18	5.25ab	6.67a	1.53b	1.99ab	0.67b	0.53	**
				Sig.	***	*	**	**	ns		
Octanal	56	1066	ms, lri, s	0	1.84	1.84	1.32	1.63	1.68	0.12	ns
				18	2.95a	2.53a	0.52b	0.21b	0.59b	0.21	***
				Sig.	ns	ns	***	***	***		
2-Octenal, (E)-	112	833	ms, lri	0	0.23	0.22	0.13	0.17	0.16	0.01	ns
				18	2.56a	1.63a	0.38b	0.28b	0.25b	0.11	***
				Sig.	***	**	***	*	ns		
1-Butanol	56	707	ms, lri	0	0.99ab	0.73ab	0.62b	0.81ab	1.12a	0.08	*

				18	2.58a	1.83b	0.81c	0.91c	0.86c	0.07	***
				Sig.	***	***	ns	ns	ns		
1-Pentanol	55	847	ms, lri	0	39.80	37.86	43.69	45.44	46.04	2.37	ns
				18	284.73a	182.26b	48.68c	41.65c	36.27c	4.54	***
				Sig.	***	***	ns	ns	ns		
1-Hexanol	56	969	ms, lri, s	0	4.26	3.48	3.02	3.80	3.27	0.15	ns
				18	1490.60a	949.67b	115.08c	100.15c	42.79c	47.30	***
				Sig.	***	***	***	***	***		
1-Octen-3-ol	57	1051	ms, lri	0	8.55ab	5.63c	6.54bc	9.17a	9.04ab	0.29	***
				18	148.20a	66.57b	25.38c	18.90c	16.21c	4.57	*
				Sig.	***	***	***	***	**		

a−c Mean values in the same row with different letters indicate significant difference between treatments; SE: standard error; Sig.: significance; n.s.: not significant; ∗ p<.05. ∗∗ p<.01 ∗∗∗ p<.001; m/z: quantification ion; LRI: Lineal Retention Index calculated for DB-624 capillary column (J&W scientific: 30 m×0.25 mm id, 1.4 μm film thickness) installed on a gas chromatograph equipped with a mass selective detector

3.7. Sensory analysis

To assess the extent to which sausages were considered acceptable, consumers assessed color, surface discoloration and odor of the raw product over time (Fig 2). On day 0, the color of all treatments was considered acceptable (scores higher than 3), wherein higher values were observed for control and E500 batches, possibly due to the higher a* values observed in these samples. Then, the scores decreased to the end of storage, when the control, C250 and C500 treatments received scores of 3. The surface discoloration was perceived as acceptable during the whole display with similar behavior among batches. Concerning the odor attribute, the control, E500 and C500 treatments were considered acceptable until day 6, whereas C250 and C750 batches were acceptable for 12 days. On day 18 all treatments were considered unacceptable in terms of odor. This finding may be related to the lipid oxidation mentioned above.

Regarding the sensory analysis performed on cooked sausages, all groups received an acceptance test score higher than 4 (acceptability limit of the hedonic scale used in this study) for all attributes. There were non-significant differences ($P>0.05$) in taste, texture and overall quality of the sausages (Fig. 3), demonstrating that the addition of turmeric extract did not affect the acceptability of these attributes on cooked lamb sausage. However, the color was influenced by the addition and concentration of turmeric extract. The control and E500 treatments received the highest scores (5.66 for both), while a gradual decrease on scores was observed as a function of extract concentration. The lowest scores (4.59) for color were observed on C750 treatment. This result is possibly related to the more yellowish color of the sausages with added turmeric extract, evidenced by the high b* values presented by these groups. However, odor scores were higher in samples containing turmeric extract (5.84, 5.72, 5.66 vs. 5.25 and 5.22 for C500, C750, C250, CONT and E500 treatmetns, respectively), which would confirm being so appreciated for its flavor in culinary applications. Therefore, this outcome indicated an improvement that may be related to the use of turmeric as ingredient in the elaboration of lamb sausages.

Figure 2. Sensory scores for color (A), discoloration (B) and odor (C) of raw fresh lamb fresh sausages (CONT − control; E500 − sodium erythorbate (500 ppm); C250, C500 and C750 − turmeric extract (250, 500 and 750 ppm, respectively) during storage. Hedonic scale used: 1 = not acceptable; 2 = hardly acceptable; 3 = acceptable; 4 = good; 5 = excellent. Error bars corresponding to standard error.

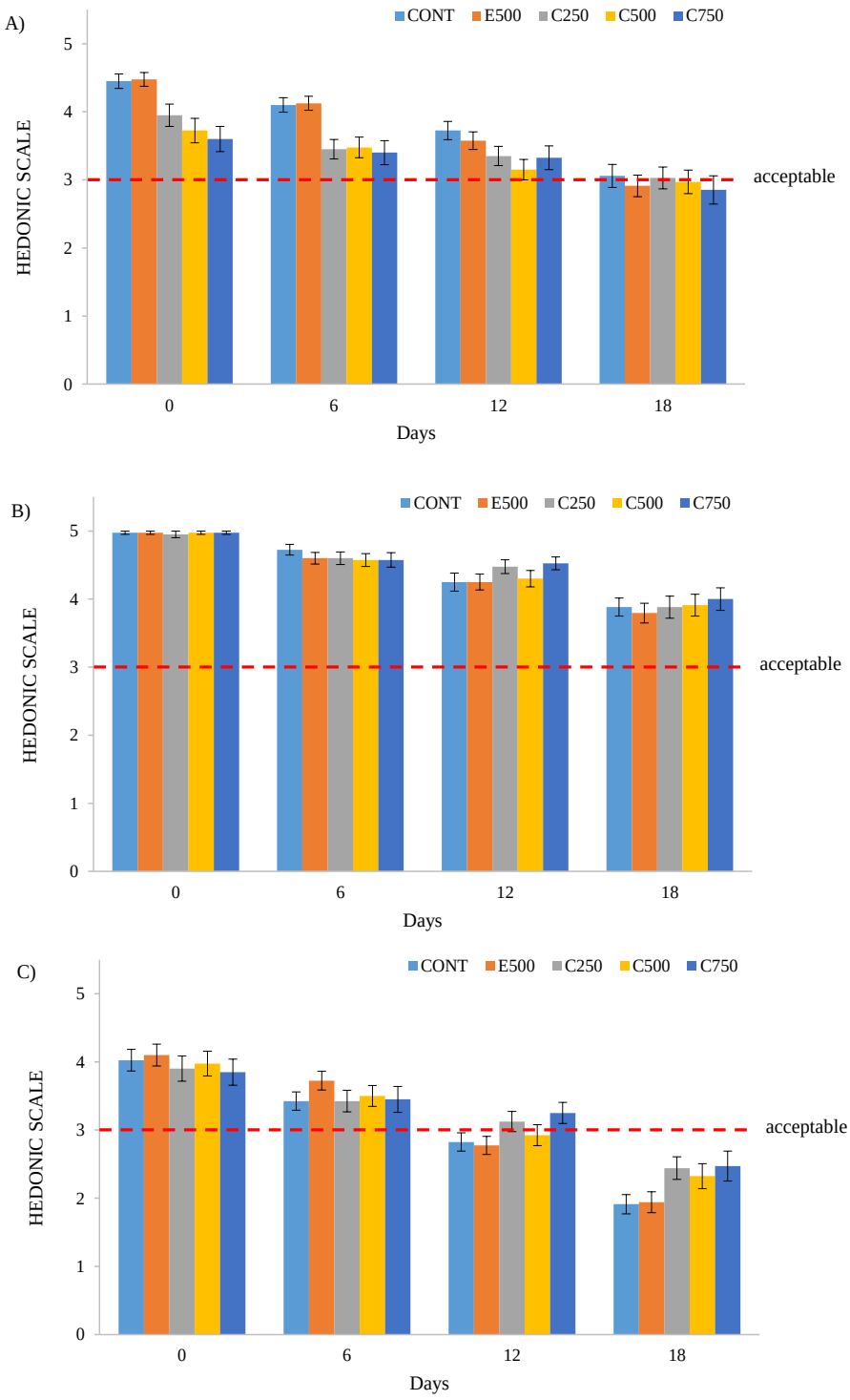
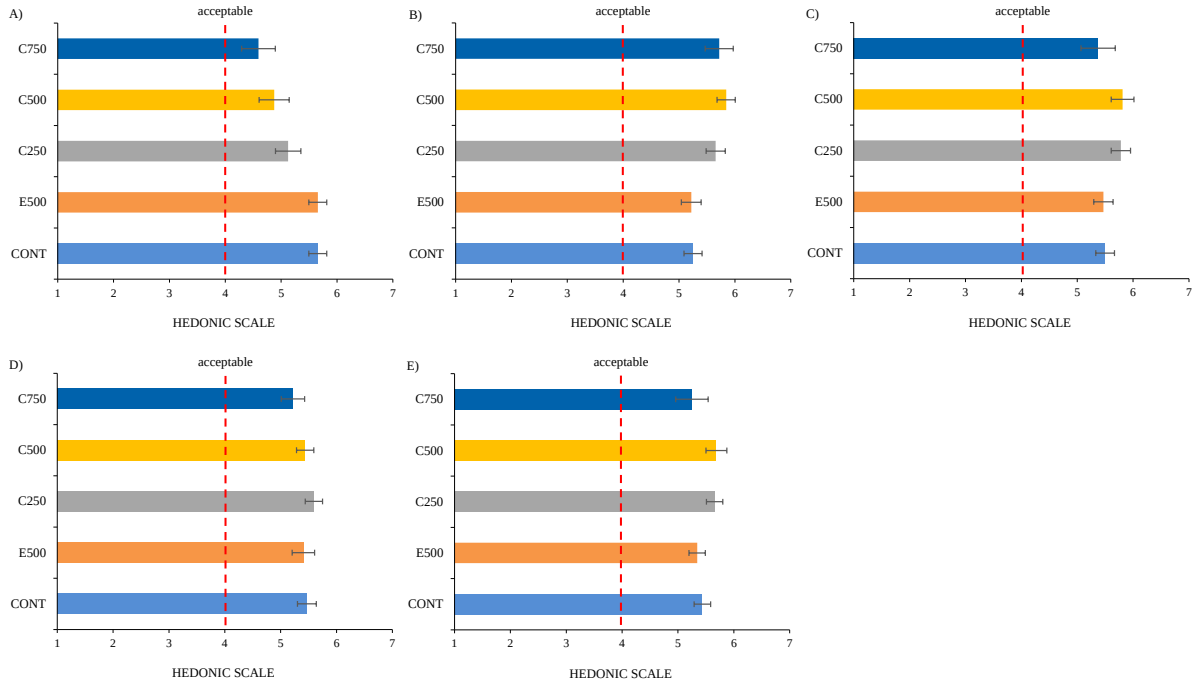


Figure 3. Sensory scores (color (A), odor (B), taste (C), texture (D) and overall quality (E)) attributed by the panelists on day 0 for cooked lamb sausages elaborated without antioxidants (control − CONT) with sodium erythorbate (E500) or turmeric extract (C250, C500 and C750). Hedonic scale used: 1 = dislike very much; 2 = dislike moderately; 3 = dislike slightly; 4 = neither like nor dislike; 5 = like slightly; 6 = like moderately and 7 = like very much. Different letters indicate significant difference between treatments (P<0.05). Error bars corresponding to standard error.



4. CONCLUSION

This study demonstrated the potential of turmeric extract to increase the shelf life of fresh lamb sausage by acting as a natural antioxidant and without affecting physicochemical properties even at lower doses than that used for synthetic antioxidant (sodium erythorbate). Turmeric extract in fresh lamb sausage showed higher antioxidant capacity throughout the storage period and consequently slowed lipid oxidation, which was confirmed by the results of TBARS assay and the lower formation of volatile compounds derived from oxidative degradation of lipids. Sausages with added turmeric extract received lower color and higher aroma scores compared to the control, but in both cases all treatments were considered acceptable by consumers. In addition, turmeric extract prolonged the acceptability of the aroma

attribute. Thus, the use of turmeric extract as a natural antioxidant to replace the synthetic antioxidant sodium erythorbate is a promising strategy to extend the shelf life and preserve quality characteristics of fresh lamb sausage.

Declarations of interest: none

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4. GENERAL CONCLUSION

The extracts of guarana seeds and pitanga leaves can replace the synthetic antioxidant in lamb hamburgers, without causing harmful changes in their physical-chemical and sensory properties. Since they suppressed the loss of redness by delaying discoloration, they showed greater antioxidant activity in protecting hamburgers against oxidation of lipids and proteins during storage time than BHT, as confirmed by the decrease in volatile compounds of lipid oxidation, more evident with extract of pitanga. In this way, guarana seeds and pitanga leaf extracts are promising natural antioxidants to increase the shelf life of meat products, even in hamburgers that are more susceptible to oxidation.

Vegetable oils provide consistent improvements in the fatty acid profile and nutritional indices of cooked lamb sausages. Sausages made with chia oil and olive oil showed a loss in texture parameters, which was observed in the sensory analysis. While linseed oil was able to improve the lipid profile without changing its technological and sensory characteristics. For this reason, among the oils tested, linseed oil is more recommended as a substitute for fat in cooked lamb sausages.

Turmeric extract has the potential to increase the shelf life of fresh lamb sausage, acting as a natural antioxidant and without harming the physical-chemical coatings, even at lower doses than the synthetic antioxidant (sodium erythorbate). The turmeric extract showed a greater antioxidant capacity during the storage period and, consequently, promoted less lipid oxidation, confirmed by the results of TBARs and less formation of volatile compounds derived from oxidation, prolonged the acceptability of the aroma attribute. Turmeric batches had a lower score for color and a higher score for aroma compared to the control, however, in both cases all treatments were considered acceptable by consumers. Thus, the use of turmeric extract as a natural antioxidant extends the shel life of meat products without compromising quality characteristics.