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Elisa Rafaela Bonadio Bellucci

Aplicação de extratos de pitaia e açaí em produtos cárneos: estudo da vida útil

São José do Rio Preto
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Tese apresentada como parte dos requisitos para obtenção do título de Doutor(a) em Engenharia e Ciência de Alimentos, junto ao Programa de Pós-graduação em Engenharia e Ciência de Alimentos (Área de concentração: Ciência e Tecnologia de Alimentos) do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de São José do Rio Preto.

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Comissão Examinadora

Profa. Dra. Andrea Carla da Silva Barretto
UNESP – Campus de São José do Rio Preto
Orientadora

Profa. Dra. Natália Soares Janzantti
UNESP – Campus de São José do Rio Preto

Profa. Dra. Marise A. Rodrigues Pollonio
UNICAMP – Campus de Campinas

Prof. Dr. Roger Darros Barbosa
UNESP – Campus de São José do Rio Preto

Profa Dra. Profa. Dra. Tayse Ferreira Ferreira da Silveira
UNIFESP – Campus São Paulo

São José do Rio Preto
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Dedico este trabalho a minha família!

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RESUMO

A mudança no hábito alimentar dos consumidores, que passaram a se preocupar mais com os alimentos que consomem, aliado aos riscos carcinogênico e tóxico associados aos aditivos sintéticos, levaram a um aumento na busca por alimentos com menores quantidades desses aditivos. Essa tendência refletiu no setor da carne impulsionando pesquisas para desenvolver produtos com adição de extratos naturais obtidos de diferentes fontes capazes de inibir ou retardar as reações de oxidação, a principal causa da deterioração desses produtos devido às alterações sensoriais e nutricionais. Extratos obtidos de frutas têm sido amplamente estudados. A pitaia vermelha e o açaí são frutos ricos em compostos bioativos com ação antioxidantes e com potencial para estender a vida útil de produtos cárneos, reduzindo as reações de oxidação lipídica e proteica, além de minimizar a descoloração durante o armazenamento. Assim, o objetivo deste trabalho foi obter extrato de pitaia vermelha para aplicação em hambúrguer suíno e avaliar os seus efeitos sobre a vida útil. Ainda neste contexto, obter extrato a partir da polpa de açaí e avaliar sua aplicação em hambúrguer suíno refrigerado e em salame tipo Italiano durante o tempo de maturação. O extrato de pitaia vermelha estudado foi adicionado em hambúrguer suíno com substituição de gordura suína por uma emulsão de óleo de chufa. Os tratamentos estudados foram: CON (sem adição de antioxidante), ERY (500 $mg.kg^{-1}$ de eritorbato de sódio), PEL, PEM e PEH (250, 500 e 1000 $mg.kg^{-1}$ de extrato de pitaia, respectivamente). O extrato de pitaia vermelha teve efeito sobre a coloração do produto, mantendo a coloração de hambúrguer suíno mais estável durante os 18 dias de armazenamento refrigerado sobre incidência de luz, além disso foi capaz de reduzir a oxidação lipídica e melhorar a aceitação sensorial, principalmente do atributo de cor. O extrato de açaí foi avaliado em ambos os produtos, hamburguer e salame, nas concentrações de 250, 500 e 750 $mg.kg^{-1}$, e estas foram comparadas a um controle (sem adição de antioxidante) e um tratamento com eritorbato de sódio (500 $mg.kg^{-1}$). Em hambúrguer, o extrato de açaí melhorou a estabilidade oxidativa, no entanto, as concentrações 500 e 750 $mg.kg^{-1}$ (AEM e AEH) afetaram negativamente a coloração, o que não ocorreu na menor concentração estudada (AEL, 250 $mg.kg^{-1}$), além disso, foi capaz de inibir as reações oxidativas tal como o eritorbato de sódio. Portanto, em hambúrguer suíno, o extrato de açaí adicionado a 250 $mg.kg^{-1}$ pode ser usado como antioxidante natural em alternativa ao eritorbato de sódio. Já quando aplicado em salame, foi possível observar que o extrato de açaí aplicado nas concentrações de 500 e 750 $mg.kg^{-1}$ melhoraram a estabilidade oxidativa, além de terem reduzido os valores de pH, e aumentado o

crescimento de bactérias lácticas no tempo de fermentação. Diante disto, nestas concentrações, o extrato de açaí pode ser usado como antioxidante natural para melhorar a qualidade e prolongar a vida útil do salame do tipo italiano. Portanto, nas concentrações estudadas, tanto extrato de pitaia quanto o de açaí podem ser utilizados como antioxidantes para melhorar a vida útil de produtos cárneos.

Palavras-chave: Antioxidantes naturais. Aditivos naturais. Produtos cárneos saudáveis. Oxidação lipídica.

ABSTRACT

The change in the eating habits of consumers, who have become more concerned with the food they consume, combined with the carcinogenic and toxic risks associated with synthetic additives, has driven an increase in the search for foods with lower amounts of these additives. This trend was reflected in the meat sector, boosting research to develop products with the addition of natural extracts obtained from different sources capable of inhibiting or delaying oxidation reactions, the main cause of deterioration of these products due to sensory and nutritional changes. Extracts obtained from fruits are widely studied. Red pitaya and açaí are fruits rich in bioactive antioxidant compounds that can act to extend the life of meat products, reducing the reactions of lipid and protein oxidation, in addition to minimizing discoloration during storage. Thus, the objective of this work was to obtain red pitaya extract for application in pork patties and to evaluate its effects on the stability of color and oxidation reactions, as well as on the sensory acceptance of this product. And, still in this context, to evaluate the effect of the extract obtained from the açaí pulp when applied in refrigerated pork patty and in Italian-type salami during the ripening time. The red pitaya extract studied was added to pork patties with pork fat replacement by an emulsion of chufa oil. The treatments studied were: CON (no antioxidant added), ERY (500 $mg.kg^{-1}$ of sodium erythorbate), PEL, PEM and PEH (250, 500 and 1000 $mg.kg^{-1}$ of pitaya extract, respectively). The red pitaya extract influenced the color of the product, keeping the pork patties color more stable during the 18 days of refrigerated storage under light incidence, in addition, it was able to reduce lipid oxidation and improve sensory acceptance, especially of the color attribute. The açaí extract was evaluated in both products, pork patties and Italian-type salami, at concentrations of 250, 500 and 750 $mg.kg^{-1}$, and these were compared to a control (without the addition of antioxidant) and a treatment with sodium erythorbate (500 $mg.kg^{-1}$). In pork patties, the açaí extract improved the oxidative stability, however, the concentrations of 500 and 750 $mg.kg^{-1}$ (AEM and AEH) negatively affected the color, which did not occur at the lowest concentration studied (AEL, 250 $mg.kg^{-1}$), in addition, it was able to inhibit oxidative reactions such as sodium erythorbate. Therefore, in pork patty, açaí extract added at 250 $mg.kg^{-1}$ cans be used as a natural antioxidant as an alternative to sodium erythorbate. When applied in Italian-type salami, açaí extract applied at concentrations of 500 and 750 $mg.kg^{-1}$ was able to improve oxidative stability, in addition to reducing the pH values and increasing the growth of lactic acid bacteria during the fermentation time. In this context, at these concentrations, the açaí extract can be used as a natural antioxidant to improve

the quality and prolong the shelf life of Italian-style salami. Therefore, at the concentrations studied, both pitaya and açai extracts can be used as antioxidants to improve the shelf life of meat products.

Keywords: Natural antioxidants. Natural additives. Healthy meat products. Lipid oxidation.

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SUMÁRIO

INTRODUÇÃO GERAL

Há um aumento do consumo de carne a nível mundial e apesar de ter sido correlacionado com algumas implicações a saúde, a carne ainda é considerada a mais importante fonte de proteínas de alta qualidade, de vitaminas do grupo B e minerais essenciais (BURRI et al., 2020; JIANG; XIONG, 2016). No entanto, carnes e produtos cárneos são acometidos de reações de oxidação devido principalmente a alta proporção de ácidos graxos insaturados e também a composição dos fosfolipídios, presença de íons metálicos, ferro presente na porção heme da mioglobina, presença de oxigênio e, devido a etapas de processamento como moagem e cozimento (BISWAS; CHATLI; SAHOO, 2012; BURRI et al., 2020; DOMÍNGUEZ et al., 2019; LORENZO et al., 2018a).

As reações de oxidação reduzem a qualidade de produtos cárneos, tendo como consequência a formação de odor de ranço, redução das características nutricionais e formação de compostos tóxicos (FAN et al., 2019). Contra esse fenômeno são adicionados aditivos a produtos cárneos com a finalidade de estender a vida útil e principalmente evitar a descoloração. Entre os antioxidantes utilizados estão o eritorbato de sódio, ascorbato de sódio, hidroxianisol butilado (BHA), butilado hidroxitolueno (BHT), terc-butilhidroquinona (TBHQ), galato de propila e sais de nitrito e nitrato de sódio e/ou potássio que se encontram associados a efeitos toxicológicos e carcinogênicos (BALDIN et al., 2018; ECHEGARAY et al., 2018; KARRE; LOPEZ; GETTY, 2013; MERCADANTE et al., 2010; RIBEIRO et al., 2019).

O risco associado aos antioxidantes sintéticos somado a mudança no habito alimentar da população mundial, que passou a se preocupar em consumir alimentos com características mais saudáveis, impulsionou a indústria a desenvolver produtos com rótulos limpos, ou seja, rótulos com ingredientes reconhecidos pelos consumidores, estimulando a pesquisa por ingredientes naturais que possam substituir aditivos químicos comumente utilizados em alimentos (BREWER, 2011; KUMAR et al., 2015). Estudos estão sendo conduzidos para investigar o efeito antioxidante de compostos extraídos de fontes naturais, avaliando sua ação na garantia da qualidade sensorial e nutricional em produtos cárneos, mostrando serem boas alternativas aos antioxidantes sintéticos devido aos altos níveis de compostos fenólicos presentes e outras substâncias antioxidantes (BALDIN et al., 2018; FAN et al., 2019; LORENZO et al., 2018a, 2018b; MUNEKATA et al., 2017; POGORZELSKA et al., 2018; RIBEIRO et al., 2019).

Neste contexto, as frutas têm se destacado como fontes de compostos bioativos com propriedades antioxidantes para aplicação em produtos cárneos. A pitaia vermelha (*Hylocereus polyrhizus*) é um fruto exótico com propriedades interessantes envolvendo sua coloração e valor nutricional, pois é rica em compostos fenólicos, vitamina C e açucares, além de possuir pigmentos como betalaínas e flavonoides, ambos com comprovada capacidade antioxidante (FERRERES et al., 2017; HUA et al., 2018; OTÁLORA et al., 2019). As betalaínas são responsáveis pela cor rosa da polpa e da casca desses frutos, são pigmentos solúveis em água que possuem nitrogênio em sua estrutura (GARCÍA-CRUZ et al., 2017). Alguns estudos mostram efeito da betalaína como corante em produtos cárneos com reduzido teor de nitrito (BELLUCCI et al., 2021; BLOUKAS; ARVANITOYANNIS; SIOPI, 1999; MARTÍNEZ et al., 2006).

A *Euterpe oleracea* é uma palmeira nativa da floresta Amazônica e é mais conhecido como açaizeiro e seu fruto, o açaí, possui alto potencial econômico devido a comercialização de seu suco e polpa, os quais são reconhecidas fontes de energia (BRUNSCHWIG et al., 2016). No Brasil, os maiores produtores de açaí são os estados do Pará, com produção anual de mais de 1,3 milhão toneladas, seguido do Amazonas e de Roraima, que juntos somam mais de 55 mil toneladas (ABRAFRUTAS, 2019). Antocianina é o principal bioativo presente no açaí, sendo associada com inativação de radicais livres e inibição da oxidação, podendo ser utilizado como antioxidante (CARVALHO et al., 2017; KANG et al., 2011). Ainda não há estudos sobre a aplicação de extrato de polpa de pitaia vermelha e de polpa de açaí em produtos cárneos como potencial antioxidante natural.

APRESENTAÇÃO DO TRABALHO

Este trabalho foi organizado em quatro capítulos para a melhor distribuição e entendimento dos assuntos abordados.

O capítulo 1 consiste em uma revisão bibliográfica sobre o tema abordado na tese. A revisão intitulada de "*Addition of natural extracts with antioxidant function to preserve the quality and shelf life of meat products*" trata-se do levantamento bibliográfico sobre o uso de aditivos naturais em produtos cárneos como alternativa a antioxidantes químicos. Este capítulo foi submetido à Revista *Food Reviews International*.

O capítulo 2 trata-se do artigo científico "*Red pitaya extract as natural antioxidant in pork patties with total replacement of animal fat"* publicado na Revista *Meat Science* de autoria de Elisa Rafaela Bonadio Bellucci, Paulo E. S. Munekata, Mirian Pateiro, José M. Lorenzo e Andrea Carla da Silva Barretto. Este capítulo aborda a obtenção de extrato a partir da polpa de pitaia vermelha e os efeitos da sua aplicação em hambúrguer suíno com substituição da gordura animal por uma emulsão de óleo de chufa sobre a estabilidade oxidativa e de cor e sobre a aceitação sensorial.

O capítulo 3 trata-se do artigo "*Açaí extract powder as natural antioxidant on pork patties during the refrigerated storage*" publicado na Revista *Meat Science*, de autoria de Elisa Rafaela Bonadio Bellucci, João Marcos dos Santos, Larissa Tátero Carvalho, Taís Fernanda Borgonovi, José M. Lorenzo, Andrea Carla da Silva Barretto. Este capítulo aborda a obtenção de extrato a partir da polpa açai e os efeitos da sua aplicação em hambúrguer suíno sobre a estabilidade oxidativa e de cor durante armazenamento refrigerado.

O capítulo 4 trata-se de um artigo elaborado para ser submetido na Revista *Meat Science* e aborda os efeitos da aplicação do extrato obtido a partir da polpa açaí em salame tipo Italiano sobre a estabilidade oxidativa e de cor durante a etapa de processamento (maturação). Este artigo foi intitulado "*Application of açaí extract powder as natural antioxidant in Italian-type salami under ripening conditions: evaluation on physicochemical and microbiological properties*" e é de autoria de Elisa Rafaela Bonadio Bellucci, Larissa Tátero Carvalho, João Marcos do Santos, José M. Lorenzo e Andrea Carla da Silva Barretto.

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OBJETIVOS

a) Objetivo Geral

Este estudo teve por objetivo avaliar o efeito da adição de extratos naturais obtidos da polpa da pitaia vermelha e da polpa do açaí sobre as propriedade físico-químicas como cor, oxidação lipídica e as propriedades sensoriais em hambúrguer suíno durante o armazenamento refrigerado em salame tipo Italiano durante o processamento (maturação).

b) Objetivos Específicos

- Produzir extratos aquosos a partir da polpa de pitaia e do açaí.
- Determinar a quantidade de compostos fenólicos totais e a atividade antioxidantes dos extratos obtidos através das análises FRAP e DPPH.
- Aplicar o extrato de pitaia em hambúrguer suíno com substituição total de gordura suína, e adição de emulsão de óleo de chufa (*Cyperus esculentus*) embalado em atmosfera modificada armazenado sob refrigeração e avaliar seu efeito sob a estabilidade de cor, oxidação e a aceitação sensorial
- Aplicar o extrato de açaí em hambúrguer suíno armazenado sob refrigeração e avaliar seu efeito sob a estabilidade de cor e oxidação lipídica.
- Aplicar o extrato de açaí em salame tipo Italiano e avaliar seu efeito durante o processamento (maturação) sob a estabilidade de cor e oxidação lipídica.

Capítulo 1

ADDITION OF NATURAL EXTRACTS WITH ANTIOXIDANT FUNCTION TO PRESERVE THE QUALITY AND SHELF LIFE OF MEAT PRODUCTS

Elisa Rafaela Bonadio Bellucc, Camila Vespúcio Bis-Souza, José M. Lorenzo, Andrea Carla da Silva Barretto

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ADDITION OF NATURAL EXTRACTS WITH ANTIOXIDANT FUNCTION TO PRESERVE THE QUALITY AND SHELF LIFE OF MEAT PRODUCTS

Elisa Rafaela Bonadio Bellucci[a], Camila Vespúcio Bis-Souza, José M. Lorenzo[b,c], Andrea Carla da Silva Barretto[a]

[a] Department of Food Technology and Engineering, UNESP – São Paulo State University, Street Cristóvão Colombo, 2265, Zip Code 15054-000, São José do Rio Preto, SP, Brazil.

[b] Centro Tecnológico de la Carne de Galicia, Avda. Galicia nº 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain.

[c] Universidade de Vigo, Area de Tecnología de los Alimentos, Facultad de Ciencias de Ourense, 32004 Ourense, Spain

*Corresponding author:

Email: andrea.carla@unesp.br

Abstract - Antioxidants are used to prevent oxidation reactions and inhibit the development of unwanted sensory characteristics that decrease nutritional quality, acceptance and shelf life of processed meat products, improving their stability. Synthetic antioxidants, although efficient, are related to the development of diseases because they present toxic and carcinogenic effects. Thus, researchers and industry study natural alternatives to synthetic antioxidants to be used in meat products, thus meeting the demand of consumers who seek foods without additives in their composition. These natural extracts have compounds that exert antioxidant activity in different meat products by different mechanisms faction. Thus, this review work aimed to gather studies that applied natural extracts derived from different plant sources or not as possible antioxidants in meat products and their action in preserving the quality of these products.

Keywords: natural antioxidants, synthetic antioxidants, bioactive compounds, lipid oxidation, meat discoloration, shelf life.

Introduction

Meat and meat products are affected by several factors during the shelf life that compromise sensory acceptance due to changes in color, odor, flavor and nutritional quality (de Carvalho et al., 2019). Changes in sensory characteristics can result in the rejection of the product and, due to the association with the freshness and safety of the product, discoloration negatively affect the purchase intention by consumers (Agregán et al., 2019).

The main cause of deterioration of products of animal origin is oxidation reactions, thus, the use of antioxidants is a way found by the industry to maintain the characteristics of fresh foods for longer by inhibiting these oxidation reactions (Domínguez et al., 2019). Chemical additives with antioxidant action are used to extend the shelf life and specially to avoid discoloration in meat products. Among the most used antioxidants are sodium erythorbate, sodium ascorbate, propyl gallate, butylated hydroxyanisol (BHA), tert-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and curing salts as nitrite and nitrate and all of them are associated with toxicological and carcinogenic effects (Baldin et al., 2018; Echegaray et al., 2018; Ribeiro et al., 2019).

It is evident that consumers are concerned about the use of chemical additives manly due its carcinogenicity, which results in an increased demand for clean label foods using only natural ingredients. In this way, the risk associated with synthetic antioxidants added to the change in the food habit of the world population, who started to worry about consuming foods with healthier characteristics, prompted the industry to look for natural additives which have greater potential for application due the consumer's acceptability in relation to synthetics (Nikmaram et al., 2018). In response to this new trend, studies are being carried out in order to find natural alternatives for synthetic additives and among the possibilities found is the use of plants extracts in meat matrices evaluating their action in maintaining sensory and nutritional quality in meat products. As these extracts contain high levels of phenolic compounds present and other antioxidant substances effective in delaying oxidative reactions (Baldin et al., 2018; Fan et al., 2019; Lorenzo, Pateiro, et al., 2018; Lorenzo, Vargas, et al., 2018; Munekata et al.,

2017; Pogorzelska et al., 2018; Ribeiro et al., 2019). Thus, this review work aimed to gather studies that applied natural extracts derived from different matrices such as fruits, spices in addition to essential oils and residues and by-products of the industry as possible antioxidants in meat products and their action in preserving quality during shelf life (Figure 1-1).

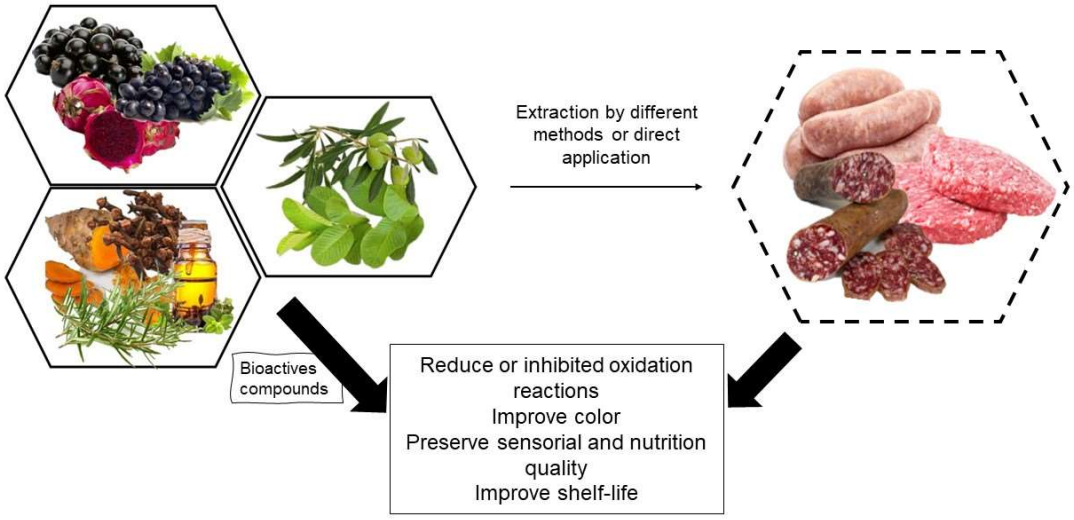


Figure 1-1 Illustration representing the application of natural sources as an antioxidant in meat products as a means of preserving quality and shelf life.

Oxidation reactions in meat and meat products

Oxidation reactions are the main non-microbial causes of meat deterioration during the storage period (Agregán et al., 2019; Cunha, Monteiro, Costa-Lima, et al., 2018) reducing its quality. The presence of unsaturated fatty acids and an increase in oxygen exposure through process steps such as milling, slicing and cooking intensifies these reactions (Lorenzo, Pateiro, et al., 2018). Oxidation reactions initially compromise the sensory quality of meat and its derivatives due the changes in texture, formation of a characteristic flavor of a rancid product and manly discoloration, that influences the acceptance by consumers. In addition, these reactions decrease the nutritional due to the loss of essential fatty acids and vitamins. However, the most impacting consequence refers to the formation of toxic products during lipid oxidation that are considered harmful to health because they are associated with some pathologies such as atherosclerosis, cancer, inflammation and aging processes, among others (Domínguez et al., 2019; Fan et al., 2019; Ribeiro et al., 2019).

The lipid oxidation process is complex and has many mechanisms that interact with each other (Domínguez et al., 2019). Figure 1-2 illustrate the mechanism of the oxidative processes that occur in the meat system. In simple terms, oxidation consists of the loss of one or more electrons by the molecule, most characterized by the loss of hydrogen and oxygen gain (Oswell et al., 2018). According to Lorenzo, Pateiro, et al. (2018) oxygen is the main factor that affects lipid oxidation, reacting with unsaturated fatty acids and producing peroxides. The oxidation process occurs in three stapes called initiation, propagation and termination (Lorenzo, Pateiro, et al., 2018; Ribeiro et al., 2019) and result in the modification of fatty acid through a self-catalytic mechanism called self-oxidation (Kumar et al., 2015).

Initiation occurs through the action of pro-oxidizing agents or reactive oxygen species (ROS) and conditions favorable to the reaction such as thermal process, presence of light, forming a fatty acid radical (R •) through the removal of the hydrogen radical. In the second stage, the propagation phase, there is the formation of peroxide radical (ROO •) through the reaction between the radical previously formed in the initiation step and the molecular oxygen (O_2). In a next reaction, primary products are formed, the so-called hydroperoxides, which are considered relatively stable products (Kumar et al., 2015). Peroxide and hydroperoxide radicals have no taste and odor. However, metal ions, light and heat cause isomerization and decomposition reactions of hydroperoxides giving rise to secondary products such as hexanal, pentanal, 4-hydroxynonenal and malonaldehyde (MDA), among others, responsible for the odor, flavor and texture characteristic of rancidity. Finally, stable or non-reactive products are formed in the termination phase from various reaction combinations that occur between free radicals or from the reaction of free radicals with non-radical compounds such as antioxidants (Domínguez et al., 2019; Ribeiro et al., 2019).

One of the most important aldehydes produced during lipid oxidation is malonaldehyde or MDA (1,3-propanedial) because it can produce rancid aroma even in small quantities and it is used to quantify lipid oxidation through analytical methods in meat and meat products. The main methods of analysis used to quantify MDA is the thiobarbituric acid (TBA) test which consists in the colorimetric measurement of the complex formed between TBA and MDA. However, there are other oxidation products that are reactive to thiobarbituric acid with color formation and, therefore, the method determines substances reactive to thiobarbituric acid (TBARs) (Domínguez et al., 2019). There are studies that relate the values of lipid oxidation between 2 and 2.5 mg MDA/kg are considers as accepted limit and still no affect the sensory acceptability of the product (Zhang et al., 2019). Other studies point out different acceptable

limits for lipid oxidation that ranged from 0.5 to 2.0 mg MDA/kg (Campo et al., 2006b; Šojić et al., 2020; Tarladgis et al., 1960).

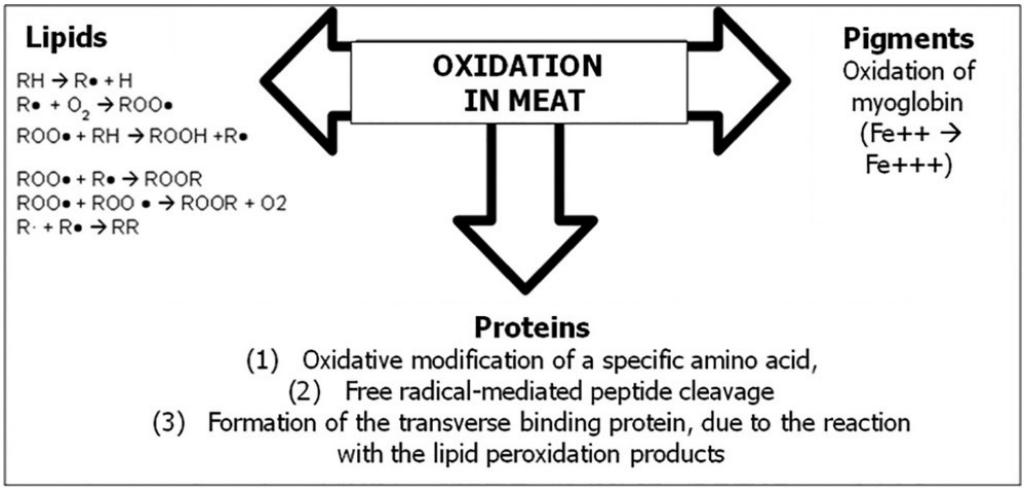


Figure 1-2 Mechanisms of the oxidative processes that occur in the meat system (Ribeiro et al., 2019).

Many components of the meat can be affected by oxidation reactions, among them proteins, which being "attacked" by reactive oxygen species lose the sulfhydryl groups, generating carbonyl compounds. The protein oxidation mechanism is considered complex, and the formation of carbonyl compounds is considered the main change in oxidized protein (Domínguez et al., 2022). There are four ways that carbonyls can form from protein oxidation, the first is when the amino acid side chain is directly oxidized, the second way is the peptide structure fragmentation, the third is when protein and reducing sugars react to each other and, at least, when a bond between protein and non-protein carbonyl compounds occur (Domínguez et al., 2022). In meat and meat products, the analytical method most used to measure protein oxidation is based on the spectrophotometric quantification of carbonyl compounds during the conversion of 2,4 dinitrophenylhydrazine to hydrazones (Lorenzo, Pateiro, et al., 2018).

Protein contributes to important technological, nutritional and sensory properties, so it is considered the main constituent of meat. Modification in its structures results in protein denaturation or proteolysis, affecting such meat properties (Domínguez et al., 2022). Due to protein oxidation, meat systems become more susceptible to the action of proteolytic enzymes, in addition to undergoing polymerization of proteins producing soluble aggregates that can cause gelation and emulsification, or insoluble aggregates that prevent binding with water

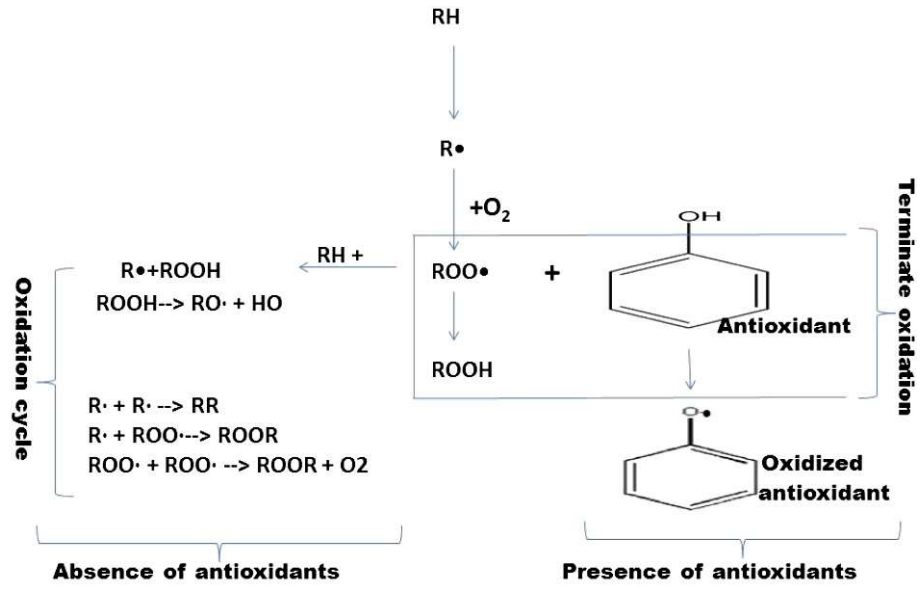
affecting its solubility. Protein oxidation can also alter its digestibility, affecting nutritional quality (Jiang & Xiong, 2016).

Finally, the impact caused by protein oxidation that most affect the quality of meat and meat products is the color changes, more specifically called pigment oxidation (Domínguez et al., 2022). Myoglobin, a protein and a pigment of the meat, is formed by a protein (globin) and a non-protein prosthetic group (heme) that contains an iron molecule. and the color variation of this pigment depends on the oxidative state that the iron. Myoglobin is purplish red in color, however it becomes bright red in the presence of high oxygen concentration, as it has become oxymyoglobin and the iron ion has become reduced (Fe^{++}). It is concluded that the partial pressure of O_2 is responsible for the oxidation states of myoglobin and that a low partial pressure favors the formation of deoxymyoglobin, an unstable pigment, which in the presence of oxygen and through the action of free radicals, becomes metmyoglobin, brown in color and iron is in oxidized form (Fe^{+++}) (Lorenzo, Pateiro, et al., 2018; Ribeiro et al., 2019).

Therefore, from a commercial point of view, with color being the sensory attribute that most influences the consumer's purchase intention (Bellucci, Barretto, et al., 2021; Ruiz-Capillas et al., 2015), oxidation reactions increase the possibility of product rejection. Therefore, the shelf life of meat and meat products consists of the moment when the consumer can detect the oxidation products that confer the rancid characteristic (mainly volatile) or observe changes in the color of the meat (Domínguez et al., 2019).

Antioxidants used in meat products

Antioxidants are used by the industry as the main strategy to decrease the intensity of oxidation reactions in meats and their processed derivatives, increasing the useful life of these products (Cunha, Monteiro, Lorenzo, et al., 2018; Domínguez et al., 2019). Synthetic compounds with phenolic structure capable of acting through the elimination of peroxyl radicals or canceling the formation of free radicals are used for this purpose (Lorenzo, Pateiro, et al., 2018). The reaction of antioxidants with oxidation is believed to occur by donating electrons to break and terminate the oxidation at the propagation step and thereby preventing additional lipid and protein radicals from forming (Falowo at al., 2014) as showing in Figure 1-3.



RH- unsaturated fatty acid, R- Free Radical

Figure 1-3 Mechanism of antioxidant in lipid oxidation at propagation step (Falowo at al., 2014).

In Brazil, the list of allowed antioxidants is different depending on the meat product that will be prepared, however, in general, the use of ascorbic acid and its derivatives is allowed (sodium, calcium and potassium ascorbate, erythorbate and sodium isoascorbate, among others) in the amount necessary to obtain the desired technological effect (quantum sadis) (Brasil, 2019). For specific products such as fresh, cooked and dried processed meat products and for canned meat and mixed, the use of propyl gallate (GP), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) in a maximum quantity between 0.01 and 0.02 g/100g on the fat content.

According to Ribeiro et al. (2019), it is important to know the maximum allowed limits of synthetic antioxidants that will be added to meat products because their consumption can have a toxic effect. Antioxidants such as BHA and BHT can cause DNA mutations and increase blood cholesterol levels, in defense the body produces enzymes that can destroy important compounds such as vitamin D, causing hives and dermatitis. Also, propyl gallate cause gastric irritations and allergic reactions, change in vision and respiratory problems when consumed in large concentrations. Ascorbic acid, on the other hand, must be used according to Good Manufacturing Practices (GMP) to be considered a safe additive.

Nitrite and nitrate salts can also be used to reduce oxidation reactions in meat products, as these salts, in addition to their preservative and curing action, also have an antioxidant action (Bellucci, Barretto, et al., 2021). However, these salts are widely associated with an increased risk of cancer due to the formation of harmful compounds called nitrosamines through the reaction of nitrite with components present in meat such as secondary amines (Gómez et al., 2015). Although these compounds have high efficiency, even at lower concentrations, consumers and the food industry started to consider the relationship between food ingredients and health, which increased the demand and interest in natural ingredients (Bernardo et al., 2021; Cunha, Monteiro, Lorenzo, et al., 2018).

In parallel, there has been a change in the habits of the population in recent years, as they have become more food conscious, concerned with consuming healthy, affordable and good-looking foods (Rodrigues et al., 2020). Teixeira & Rodrigues (2021) also state that the way meat products are produced is a concern of consumers, so the industry started to reformulate its meat products to meet this new demand for cleaner and healthier labels by reducing fat, salt, curing salts (nitrite and nitrate) and replacing synthetic antioxidants.

In this regard, synthetic antioxidants are being rejected by consumers due its potential toxicity use of natural extracts has also been of great interest to the meat industry since they started to be considered safe for consumers (Munekata et al., 2020). So, studies are being conducted in order to find compounds obtained from plants capable of reducing oxidative reactions in meat products and replacing synthetic antioxidants (Pateiro, Gómez-Salazar, et al., 2021). In addition, such replacement has been widely accepted by consumers (Munekata et al., 2017).

To be considered as antioxidants, natural extracts cannot negatively interfere with sensory characteristics, act at low concentrations, must be cheap and stable over time and storage conditions and, most importantly, cannot be harmful or toxic in the concentrations used (Munekata et al., 2020). Different plants are being tested in order to investigate their action as antioxidants in meat and meat products and extracts are obtained from different sources such as pulp, peel and seeds of fruits, spices and essential oils, as well as other sources such as flowers, nuts, leaves, vegetable residues, etc. (Basanta et al., 2018; Bellucci et al., 2022; Bellucci, Munekata, et al., 2021; Burri et al., 2020; Dadi et al., 2019; Khemakhem et al., 2019).

The mechanism of action of natural antioxidants is different depending on the compound. In general, they antioxidant effect is associated with their eliminating reactive

species capability, curbing autoxidation by donating a hydrogen atom to stabilize the first oxidation products, their potential chelation of metal ions (pro-oxidants) and their role as inhibitory of the hydro peroxide's degradation (Nikmaram et al., 2018). Phenolic compounds are the main natural antioxidants, among them the most important are tocopherols, flavonoids and phenolic acids. These compounds have a strong ability to donate hydrogen and a free radical scavenging capability (Kumar et al., 2015; Munekata et al., 2021; Nikmaram et al., 2018). According to Oswell et al. (2018), the compounds that have antioxidant activity in meat products are carotenoids, hydroxycinnamic acid, flavonoids, terpenes and antioxidant vitamins.

Antioxidant substances derived from fruits (pulp, pigment, peel and seeds) applied in meat products

Studies using fruit or extracts derived from fruits as a source of antioxidant compounds have been widely carried out in meat products, as many works have been published in recent years (Baldin et al., 2016, 2018; Basanta et al., 2018; Bellucci, Barretto, et al., 2021; Bellucci et al., 2022; Cunha, Monteiro, Costa-Lima, et al., 2018). Fruits are rich sources of bioactive compounds and pigments such as phenolic compounds, including anthocyanins and catechins, in addition to carotenes, betacyanin that have high antioxidant capacity. Table 1-1 shows studies and results about the application of substances derived from fruits (pulp, pigment, peel and seeds) applied in meat products

Some works evaluated pink guava fruit as natural antioxidant in meat products. In this regard, Chang et al. (2020) observed that flesh of this fruit presented an important antioxidant activity for containing large amount of vitamin C and phytochemicals as lycopene, carotenoid, and flavonoids. Also, the phenolic compounds like apigenin, myricetin, anthocyanins, and ellagic acid contribute with the ascorbic acid to its antioxidant activity. Therefore, the antioxidant action of pink guava pulp was evaluated by Joseph et al. (2014a) in raw pork emulsion for nine days in refrigerated storage and aerobic packaging. The authors found that guava pulp improved the color stability during storage and stated that the increase in added concentration resulted in an increase in a* values and correlated this effect to the lycopene present in the emulsion (1.9 *vs*. 4 .4 $mg.kg^{-1}$ in 0 day and 1.7 *vs*. 3.5 $mg.kg^{-1}$ in 9 storages for 5 *vs*. 10%). Regarding the antioxidant effect, they observed that the pink guava pulp was able to minimize lipid oxidation, showing higher values of TBARS for the control without addition of pulp and the values were reduced according to the increase in the added concentration of the

pink guava pulp. Pink guava pulp was also studied, in addition to tomato-derived products (tomato puree, freeze-dried tomato peel and tomato pulp), by Joseph et al. (2014b) in pork emulsified products (Table 1-1). In this study, the tested antioxidants improved the color, reduced the formation of metmyoglobin and minimized lipid oxidation in raw meat emulsion, at the concentrations studied.

According to Domínguez et al. (2020), tomato by-products such as seed, skin, pulp residues are rich in a variety of compounds with antioxidant and coloring properties, such as carotene (lycopene, beta-carotene, phytoene, phyto fluorine and lutein), phenolic compounds (phenolic acids and flavonoids) and vitamins A and C. In addition, García-Cruz et al. (2017) affirmed that phenolic compounds are photochemical associated with beneficial effects to the body, which may have a cardio protective, antioxidant, antimicrobial, ant allergic action and act against thrombosis. Moreover, Mercadante et al. (2010) investigated the effect of natural pigments (norbixin, lycopene, zeaxanthin and β-carotene) in refrigerated sausage (Table 1-1) and reported that the dyes improved the oxidative stability of the product, wherein zeaxanthin and norbixin were the most efficient.

Basanta et al. (2018) prepared and extracted fiber microparticles from the pulp and skin of Japanese plum by extraction with ethanol and then freeze dried to be added to breast chicken patties (Table 1-1). According to the authors, the microparticles retain carotenoids, tocopherol and phenolic compounds (pentameric proanthocyanidins) and the cyanidin present in high amounts is responsible for the intense red color of the fiber. The microfiber obtained from the pulp was able to improve the color within 10 days of refrigerated storage and both (added at 2.0%) reduced lipid oxidation around 50%.

Pomegranate peel as phenolic compounds source: Advanced analytical strategies and practical use in meat products, the pomegranate peel represents 40 to 50% of the total weight of the fruit and, being rich in phenolic compounds, it is a valuable residue of the industry. Pomegranate peel has considerable amounts of phenolic compounds, including flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolysable tannins, as well as phenolic and organic acids, which gives them an important antioxidant, antimicrobial, antifungal and coloring action (Smaoui et al., 2019). Turgut et al. (2017) investigated the antioxidant action of an aqueous and freeze-dried extract obtained from pomegranate peel (0.5 and 1%) in frozen beef meatballs (Table 1-1). The extract added in concentration not only kept the color stable for 6 months of storage, but also delayed lipid and protein oxidation without impairing sensory acceptance.

Table 1-1 Fruit (pulp, pigment, peel and seeds) as a source of antioxidants applied in meat products.

Source or active compound	Levels added	Sample	Storage conditions	Effect	Reference
Pink guava pulp (PGP) (ß-carotene and lycopene)	5%, 7.5% and 10%	Raw pork emulsion	Nine days in refrigerated storage and aerobic packaging	Increase redness (improves color) Reduces metmyoglobin formation High inhibition of lipid oxidation at 10%	Joseph et al., 2014a
Tomato products and PGP	Tomato puree - 10%, tomato pulp - 12.5%, lyophilized tomato peel -6 %, and PGP - 10 %)	Pork emulsion	9 days at 4±1 °C in darkness and aerobic conditions	Improves color and reduce lipid oxidation	Joseph et al., 2014b
Natural pigments: Norbixin, lycopene, zeaxanthin, ß-carotene	0.05 g/100 g	Sausage	Vacuum package and under refrigeration (4 °C) for 45 days	Norbixin and zeaxanthin presented the lower lipid oxidation values after 45 days of refrigerated storage.	Mercadante et al., 2010
Plum (*Prunus salicina*) peel and pulp micro particles	2.0 % w/w level	Breast chicken patties	Polyethylene films (oxygen permeability= 5.20×10^{-12} $m^3\cdot m^{-2}\cdot s^{-1}\cdot Pa^{-1}$); dark, at 4.0 ± 0.5 °C for 10 days	Improve color end reduced lipid oxidation around 50% during 10-days storage	Basanta et al., 2018

Source or active compound	Levels added	Sample	Storage conditions	Effect	Reference
Red pitaya extract	500, 750 and 1000 $mg.kg^{-1}$	Pork patties with replacement of animal fat	Modified atmosphere (80% O_2 and 20% CO_2); fluorescent light during 18 days at 2 ± 1 °C	Did not affect cooking loss and texture profile; increased redness and improve color stability; Reduce lipid oxidation and improve sensorial scores	Bellucci et al., 2021
Açaí extract powder	250, 500 and 750 $mg.kg^{-1}$	Pork patty	Packed in nylon-polyethylene bags without vacuum during 10 days in the dark at 2 °C	Reduced lipid oxidation; not affect pH, cooking parameters (weight loss and shrinkage); 500 and 750 $mg.kg^{-1}$ compromised color parameters; 250 $mg.kg^{-1}$ was effective as natural antioxidant	Bellucci et al., 2022
Microencapsulated extract of pitaya (*Hylocereus c*.) peel	100 and 1000 ppm	Pork patty submitted to high pressure processing	9 days of storage at 4 °C under aerobic packaging	Able to reduce protein oxidation after 9 days of storage; not affect lipid oxidation	Cunha et al., 2018
Microencapsulated jaboticaba *(Myrciaria cauliflora*) extract	2 and 4%	Fresh pork sausage	Dark and aerobic condition for 15 days under refrigeration (1 ± 1°C)	Reduce lipid oxidation; 4% affect color, texture and overall acceptance of the pork sausages; 2% not affect global acceptance and color	Baldin et al., 2016
Lyophilized powder of pomegranate peel extract	0.5 and 1.0% PE	Beef meatballs	Oxygen-permeable bags; frozen storage at−18±1 °C for 6 months	Increase L* values; reduce discoloration (higher values of a* at the end of storage); reduce protein and lipid oxidation	Turgut et al., 2017

Source or active compound	Levels added	Sample	Storage conditions	Effect	Reference
Dry red grape pomace	1 and 2 %	Meat emulsion system/cooked sausage	Vacuum package and storage at 4 °C	Affected color; not compromise sensory acceptance; increase the oxidative stability	Riazi et al., 2016
Grape seed extract	100, 300 and 500 ppm	Beef sausage model system	Package in PVC; frozen at -18 °C for 4 months	All concentrations protected the meat product model system against lipid oxidation	Kulkarni et al., 2011
	1000 $mg.kg^{-1}$	Pig liver pâté	Packed in glass containers (150 g); cooked; stored in the dark at 4°C for 24 weeks	Reduced lipid oxidation better than BHT	Pateiro et al., 2014
Guarana seeds extract	250, 500 and 1000 mgkg	Pork patty	Modified atmosphere (80% O_2 and 20% CO_2); under light during 18 days at 2 ± 1 °C	Shown higher inhibitory effect on oxidation reactions (lipidic and protein) than BHT at 250 and 500 $mg.kg^{-1}$; improvement of product color	Pateiro et al., 2018
Rosemary, acerola and citrus (lemon, orange and buffered vinegar) extracts	Rosemary extract -0,3%; acerola extract – 0,25%; citrus extract – 0,6%	Beef patty	Packaged in polystyrene trays lined with an oxygen permeable PVC film and displayed simulating retail conditions during 6 days at 4 ± 1 °C	Citrus extract inhibited the lipid oxidation during all display period and reduced the aerobic plate count; citrus and acerola improved patty color	Fruet et al., 2019

In a study, Riazi et al. (2016) showed that the addition of red grape pomace powder in beef sausage emulsion reduced the lipid oxidation in the product during refrigerated storage compared to the control, and that, despite making the samples darker, sensory acceptance was not compromised. Also in this study, the authors stated that the grape pomace powder had the potential to be used as a healthy ingredient and to act in the oxidative stability of meat products. Grape pomace is a residue from the production of grape juice consisting of skin, seed and stem that contains high amounts of polysaccharides and phenolic compounds, which are mostly anthocyanins, catechins, procyanidins and phenolic acids.

Grape seed can be considered an alternative to preserve oxidative stability in meat products because it has a high content of polyphenolic compounds and proanthocyanidins and mainly because it presents benzoic acids (gallic acid and protocatechuic acid), flavan-3-ols monomer and oligomeric procyanidins as main compounds (Pateiro et al., 2014). Kulkarni et al. (2011), for example, studied the effect of grape seed extract on pre-cooked, frozen, and re-heated beef sausage model system. In this study, the extract was added at concentrations of 100, 300 and 500 $mg.kg^{-1}$ and compared to a control, ascorbic acid, and propyl gallate, both added at a concentration of 100 $mg.kg^{-1}$. The authors found that the extract used at the lowest concentrations showed similar results to propyl gallate for oxidative, color and sensory stability in the 4 months of storage. In pork liver pate, the addition of grape seed extract (1000 $mg.kg^{-1}$) had a better effect on lipid oxidation than the treatment added with BHT (200 $mg.kg^{-1}$) during storage at 4 °C for 24 weeks, confirming its antioxidant capacity in meat products (Pateiro et al., 2014).

In pork patties, the effect of adding extract of guarana seeds was evaluated by (Pateiro, Vargas, et al., 2018). In this work, the patties were evaluated for 18 days of storage at 2 ± 1 °C (Table 1-1) and it was observed that the guarana seed extract at both concentrations (250 and 500 $mg.kg^{-1}$) not only reduced lipid oxidation when compared to BHT (200 $mg.kg^{-1}$) as well as improved color, lipid, and protein stability. It should also be noted that the guarana seed extract has many phenolic compounds, with tyrosol and other low molecular weight phenolics appearing in greater quantities.

An interesting approach was tested to improve the stability of beef patty during storage (6 days at 4 °C under retail conditions). With the purpose of evaluating the effect of a new combination of extracts, Fruet et al. (2019) developed an antioxidant with lemon, orange and buffered vinegar and compared it with acerola and rosemary extracts in beef patties. Although rosemary and acerola extract (0.30 and 0.25 %) were similar in relation to antioxidant activity,

there was an increase in lipid oxidation products during storage. However, the proposed extract avoided the formation of these lipid oxidation products in the concentration used (0.6%), inhibited the increase in TBARS values during storage. Moreover, it was efficient inhibited de growing of aerobic bacteria during storage due the buffered vinegar in this extract.

Betacyanin as betanin are present in pitaya or dragon fruit, and they are known not only for their color, but also for their antioxidant activity that record up to double of some anthocyanins (Shaaruddin et al., 2017). According to Cunha, Monteiro, Costa-Lima, et al. (2018), pitaya has a large amount of beta-carotene, betacyanin, lycopene, vitamin E, vitamin C and polyphenols. In this context, a study was developed to investigate the antioxidant effect of red pitaya extract in pork patties with animal fat replacement (Bellucci, Munekata, et al., 2021). In this work, an aqueous extract and lyophilized of red pitaya pulp (250, 500 and 1000 $mg.kg^{-1}$) were added to the patties as a natural antioxidant. A control (without antioxidant) and a treatment added of sodium erythorbate (500 $mg.kg^{-1}$) was elaborated. The authors observed a reduction in discoloration over time due to pitaya extract. Regarding oxidative stability, the extract was effective in reducing lipid oxidation reactions and carbonyl formation compared to the control without the addition of antioxidants.

Another extract that has been studied for application in meat products was obtained from açai palm fruit (*Euterpe oleracea* Mart.). This fruit is natural from Amazon region and is widely known for having high antioxidant activity *in vitro* due its rich composition in phenolic compounds such as different anthocyanins, flavones, and phenolic acids (Gordon et al., 2012). Therefore, Bellucci et al. (2022) applied an extract obtained from the açaí pulp in pork patty to evaluate its antioxidant activity (Table 1-1). The results of this study showed that açaí extract reduced lipid oxidation in pork hamburger as much as sodium erythorbate.

However, bioactive compounds derivate of plant are instable chemically and can undergo to oxidative degradation by exposure to oxygen, light, metal ions, pH and other parameters, and their direct incorporation in food is very restricted (Baldin et al., 2016; Smaoui et al., 2021). In this regard, microencapsulation is an innovative technique that can be used to preserve the bioactive present in plant extracts, enabling greater stability and preserving their functional quality (Cunha, Monteiro, Costa-Lima, et al., 2018; Munekata et al., 2020; Shaaruddin et al., 2017; Smaoui et al., 2021). Studies were performed to investigate the preservation of bioactive by a microencapsulation (Dadi et al., 2019; do Valle Calomeni et al., 2017; Ghaderi-Ghahfarokhi et al., 2017; Leyva-Porras et al., 2021; Sharayei et al., 2020; Taofiq et al., 2019). On the other hand, some authors investigated the use of microcapsules containing

bioactive compounds in meat products as alternative to synthetic antioxidants (Baldin et al., 2018; Cunha, Monteiro, Costa-Lima, et al., 2018; Surendhiran et al., 2020; Tometri et al., 2020).

In this regard, Cunha, Monteiro, Costa-Lima, et al. (2018) evaluated the action of microencapsulated extract from pitaya peels on pork hamburger submitted to a high-pressure process. The process causes protein denaturation and membrane damage, releasing non-heme iron, and consequently increasing lipid oxidation. The authors reported that the microencapsulated extract from pitaya peels (100 and 1000 $mg.kg^{-1}$), despite not having reduced lipid oxidation as much as BHT (100 $mg.kg^{-1}$), was able to minimize it and was also able to reduce protein oxidation after 9 days of refrigerated storage in an emulsified pork product subjected to a high-pressure process. Also, the extract rich in anthocyanin (cyanidin-3-o-glucoside) obtained from Jaboticaba peel was microencapsulated by spray-dryer into maltodextrin to be applied in fresh pork sausage as natural antioxidant (Baldin et al., 2016, 2018). In this work, jaboticaba extract microencapsulated were added at the concentrations of 2 and 4% reduced lipid oxidation during 15 days of refrigerated storage, presenting TBARS values lower than the other treatments (cochineal carmine and control) (Baldin et al., 2016). Still on this study, the extract (2%) did not affect the global acceptance of fresh sausage, showing itself to be an alternative as a natural coloring agent.

Spices and essential oils as naturals source of antioxidants applied in meat products

Herbs and spices or products derivates from them have been used to preserve food to be considered as natural antioxidant, mainly in meat products and its use is beneficial to prevent the oxidation reactions and develop clean label products, desirable for consumers (Khanam & Prakash, 2021). Table 1-2 shows studies and results about the application spices and essential oils as naturals source of antioxidants in meat products.

Many studies have been conducted in recent years to investigate its effect on the stability of lipid oxidation, protein and color in meat products. For example, the aqueous extract of Dog rose *(Rosa canina* L., RC) was tested by Vossen et al. (2012) in porcine sausage (frankfurter) for presenting relevant amounts of phenolic compounds, such as procyanidins and catechins, and ascorbic acid. The concentrations of 5 and 30 g/kg of extract were compared to both positive control treatment added to ascorbic acid (0.5 g/kg) and sodium nitrite (0.1 g/kg) and a negative control without the addition of any antioxidant. In this study, dog rose extract decreased the formation of lipid oxidation products as much as the combination of sodium nitrite and ascorbic

Table 1-2 Spices, products derivates from them and essential oil as naturals source of antioxidants applied in meat products

Source or active compound	Levels added	Sample	Storage conditions	Effect	Reference
Dog rose (*R. canina* L.) extract	5 and 30 $g.kg^{-1}$	Porcine sausage (frankfurter)	Oxygen permeable poly-vinyl chloride film; dispensed in polypropylene trays; stored for 60 days at 2 °C in the dark	Both concentrations inhibited lipid and protein oxidation	Vossen et al., 2012
Rosemary extract	0, 250, 500 and 750 $mg.kg^{-1}$	Reduced nitrite cooked liver pattè	Seven days in the dark at 4 °C;	Significantly reduced the lipid oxidation. However, it shows no effect on color stability.	Doolaege et al., 2012
	1500 and 2500 ppm	Raw frozen and cooked-frozen pork sausage	Packed in high-barrier film pouches; frozen in an air-blast freezer at -30 °C; stored at -20 °C.	In raw frozen: Inhibited the lipid oxidation, shown lower values than samples with BHA/BHT; improve and preserved the color. Cooked-frozen: oxidation results were similar to BHA/BHT treatments.	Sebranek et al., 2005
	Ranged from 500 to 3000 ppm	Fresh-refrigerated pork sausage	Placed on foam-board trays, overwrapped with oxygen-permeable film and stored on shelves under 7.0 lux florescent lighting at 2–4°C	2500 and 3000 was effective in inhibiting lipid oxidation such as the use of BHA/BHT;	Sebranek et al., 2005

Source or active compound	Levels added	Sample	Storage conditions	Effect	Reference
Turmeric (*Curcuma longa* L.) extract	250, 500 and 750 $mg.kg^{-1}$	Fresh lamb sausage with fat replacement by Tiger nut (*Cyperus esculentus* L.) oil	Package 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, stored at 2 ± 1 °C under light for 18 days.	750 $mg.kg^{-1}$ shown the highest antioxidant activity throughout the storage period; reduced lipid oxidation;	de Carvalho et al., 2020
Turmeric and black pepper spices	For 250 g of raw patty: turmeric – ranged from 0 to 6 g; black pepper – ranged from 0 to 1,1 g.	Cooked meat patties	Stored at -20ºC until tested.	A decrease in lipid peroxidation.	Zhang et al., 2015
Pink pepper extract	Pink pepper extract concentration equivalent to 90 mg GAE/kg meat	Chicken burger	Two different conditions: aerobic packing (PVC film) and vacuum; stored at 2 °C with white light incidence; evaluated for consecutive 7 days	Pink pepper extract reduced oxidative products as BHT synthetic antioxidant and improved color (presented higher redness). Regarding to packing condition, no effect was observed to pink pepper and BHT treatments on lipid oxidation.	Menegali et al., 2020

Source or active compound	Levels added	Sample	Storage conditions	Effect	Reference
Clove extract	0.1 %	Cooked beef patties	Packaged in a polyethylene pack and storage under refrigerated conditions for 10 days	The clove extract was more efficient to reduce lipid oxidation in cooked beef patties than BHT (0,02%) and ascorbic acid (0,05%); more efficient in preserve protein oxidation than BHT and similar to ascorbic acid at 10 days	Zahid et al., 2020
Rosemary essential oil	150, 300 and 600 ppm	Different types of frankfurters: produced with tissues from Iberian pigs (IF) or white pigs (WF)	Storage in the dark for 60 days at 4 °C.	IF: 300 and 600 ppm was effective to reduce lipid oxidation and 600 ppm reduce hexanal formation. WF: 300 and 600 ppm increased lipid and protein oxidation	Estévez & Cava, 2006
	0.2 %	Poultry fillets	Two different conditions: air-packaging and modified atmosphere	The combination of rosemary essential oil and modified-atmosphere packaging reduced the level of lipid oxidation.	Kahraman et al., 2015
Blend of essential oils (garlic, cinnamon, cloves and rosemary); Rose hips extract	1 g/kg of meat; 300 mL/kg of meat	Iberian cooked hams	Vacuum-packed and storage for five months under refrigeration conditions (4 °C)	The essential oils blend was more effective against lipid oxidation than rose hips extract and commercial antioxidant. Whereas rose hips presented better results regard the protein oxidation.	Armenteros et al., 2016

Source or active compound	Levels added	Sample	Storage conditions	Effect	Reference
Essential oils: clove; holy basil; cassia and thyme oil	clove oil (0.25%), holy basil oil (0.125%), cassia oil (0.25%) and thyme oil (0.125%)	Fresh chicken sausage	Packed in vacuum and stored at 18 ± 2 ° C for 45 days	Clove oil was the most effective regarding reduce lipid oxidation. All essential oil presented antioxidant action and reduced discoloration during storage.	Sharma et al., 2017
Satureja montana L. essential oil	7.80, 15.60 and 31.25 µl/g.	Mortadella-type sausage with different concentration of sodium nitrite	Storage at room temperature (25 °C) for 30 days.	Color was negatively affected by addition of high concentrations of essential oil; essential oil at 15.60 µl/g with sodium nitrite (100 $mg.kg^{-1}$) presented the best results regarding reduce lipid oxidation	Coutinho de Oliveira et al., 2012

acid, however, it was shown to be more efficient in inhibiting protein oxidation during the 60 days of refrigerated storage (2 °C). In addition, the authors report a possible contribution of the extract in the pink sausage coloration.

The antioxidant effect of rosemary extract has been demonstrated in many studies in different products such as hamburgers, sausages, fresh and cooked sausages (Fruet et al., 2019; Gao et al., 2019; Heck et al., 2018; Schilling et al., 2018). Doolaege et al. (2012), for example, investigated the action of different concentrations of rosemary extract (0, 250, 500 and 750 $mg.kg^{-1}$) on lipid oxidation and color stability in liver pâté added from different concentrations of sodium nitrite (40, 80 and 120 $mg.kg^{-1}$). Rosemary extract contains phenolic diterpenes, such as carnosic acid and carnosol that act as hydrogenic donors in the chain reaction of free radicals, having its antioxidant action well known. What was proven in this study, since the addition of the extract provided lower values of TBARS, therefore, decreased lipid oxidation in raw and cooked liver pâté, however, no concentration effect was observed between 250 and 750 $mg.kg^{-1}$. At the same time maintained high concentrations of antioxidants ascorbic acid, α-tocooferol and carnoic acid. Regarding sodium nitrite, it was concluded that the addition of 80 $mg.kg^{-1}$ of nitrite would be sufficient when associated with the addition of the extract without impairing the oxidative and color stability of the pâté.

On the other hand, Sebranek et al. (2005) carried out two experiments under study for antioxidant efficacy of rosemary extract, the first concentrations of 1500 and 2500 $mg.kg^{-1}$ were added in frozen and precooked sausages and, the second, from 500 to 3000 $mg.kg^{-1}$ in refrigerated sausages and the addition of BHA and BHT in the proportion of 1:1 (200 $mg.kg^{-1}$) was compared. In the first experiment, rosemary extract was more efficient leaf than synthetic antioxidants, presenting better results for oxidation stability and instrumental and sensory color in raw sausage stored under freezing for 112 days. The same did not occur in cooked-frozen sausage, because the concentrations of the extract presented similar results to each other and did not differ from the treatment with BHA/BHT in relation to the antioxidant potential. Regarding the second experiment, 2500 and 3000 $mg.kg^{-1}$ of rosemary extract had a similar effect to BHA/BHT in fresh chilled sausage. In this work it was interesting to realize that the extract at 2500 $mg.kg^{-1}$ was as effective as the maximum permitted concentrations of BHA/BHT in fresh and chilled pork sausage and in the boiled frozen sausage but was superior to the BHA /BHT in raw frozen sausage, showing that applications and conditions should always be considered. Either way, rosemary extract can be used with a substitute for synthetic antioxidants.

Turmeric *(Curcuma longa* L.) is widely used as seasoning, preservative and dye in food in Southeast Asian countries, India and China. Several sesquiterpenes and curcuminoids were isolated from the turmeric rhizome, and curcuminoids were responsible by their yellow color action and equivalent between 2 and 7% of their composition (de Carvalho et al., 2020; Gul & Bakht, 2015). The condiment is obtained by boiling the roots, followed by drying, but for the study conducted by de Carvalho et al. (2020), the extract was obtained from the extraction by supercritical fluid and presented high antioxidant capacity for the extract from the DPPH and ABTS assays (42.92 mg Trolox/g and 1490.53 mg ascorbic acid/100g, respectively). When applied in lamb sausage with fat substitution by vegetable oil emulsion, the extract at the concentrations used (250, 500 and 750 $mg.kg^{-1}$) had better results in preventing lipid oxidation than sodium erythrobate (500 $mg.kg^{-1}$) during the 18 days of refrigerated storage.

Another study was conducted with the addition not only of turmeric, but also of black pepper *(Piper nigrium)* in cooked meat patties (Zhang et al., 2015). For the authors, curcuminoids exhibit high antioxidant activity due to their cityhood of trapping oxygen radicals and breaking the oxidation chain. In this study, black pepper did not present antioxidant effect, but there was a synergistic effect with turmeric, with its antioxidant activity increased when associated with aromatic constituents and terpenes present in black pepper. In a different study, pink pepper extract *(Schinus terebinthifolius* Raddi) was added to chicken burgers with antioxidant effect as much as BHT during refrigerated storage for 7 days (Menegali et al., 2020). The authors also state that pink pepper extract, which contains bioactive compounds such as ascorbic acid, phenolic and carotenoid compounds, can be used as an antioxidant in a meat product, reducing lipid oxidation, without affecting sensory attributes.

Clove is another widely studied spice as it is an aromatic condiment widely used in food and are added to increase shelf life and reduce food deterioration. It also has a variety of bioactive compounds, such as sesquiterpenes and triterpenoids and eugenol (4-alil-2-methoxyphenol), its main bioactive compound that has insecticide and antioxidant properties and is classified as a safe additive (El-Maati et al., 2016). After studying on different solvents to obtain clove extract, El-Maati et al. (2016) concluded that ethanol is more appropriate for the extraction of phenolic and flavonoid stations and that the antioxidant capacity was directly proportional to the amount of total phenolic compounds.

Zahid et al. (2020), however, used water as a solvent to obtain clove extract and applied it in cooked beef patties stored under refrigeration. Still, in 10 days of storage, the aqueous extract of clove (0.1%) showed the best antioxidant activity, as it had lower values of lipid and

protein oxidation compared to BHT (0.02%). Regarding ascorbic acid (0.05%), the extract had higher values a* and color notes in the sensory analysis preserving the color of hamburgers, in addition to lower TBARS values. All antioxidants used reduced lipid and protein oxidation, maintained better a* values and preserved the stability of sensory attributes when compared to control without added antioxidants.

The incorporation of essential oils in meat products is an alternative to preserve oxidation reactions because they are rich in bioactive compounds, such as terpenes, terpenoids and phenolics that have proven antimicrobial, antifungal, antioxidant, antiviral, antimycotic, antiparasitic and insecticide properties (Falleh et al., 2020; Pateiro, Munekata, et al., 2021). In addition, essential oils are classified General Recognized as Safe (GRAS), allowing their use in food products as safe additives, besides being widely accepted by consumers, thus generating greater interest in their application in meat products (Pateiro, Barba, et al., 2018). Ginger, oregano, rosemary, sage, thyme, mint and many other aromatic plants are the main sources of essential oils. Conventional distillation methods using steam and hydro distillation are employed, while innovative techniques are being proposed to obtain essential oil such as hydro distillation with the aid of microwaves or extraction with supercritical fluids and assisted ohmic distillation (Pateiro, Barba, et al., 2018).

Many studies were carried out in order to investigate the antioxidant and antimicrobial potential of several essential oils in meat products (Sharma et al., 2017; Smeti et al., 2018; Vieira et al., 2019). According to Pateiro, Barba, et al. (2018), the bioactive present in the essential oils responsible for antioxidant activity are alcohols, aldehydes, phenylpropanoids, terpenes and keones, while compounds such as thymol, cymenone p, γ-terpineno and carvacrol are related to their antimicrobial activity and may act against various bacteria including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Clostridium perfringens*, *Clostridium sporogeneses* in various meat products.

Estévez & Cava (2006) studied the effect of rosemary essential oil on lipid and protein oxidation in several types of frankfurters, produced with Iberian pig and white pig. In white pig frankfurters, 300 and 600 ppm reduced TBARS values, and the highest level also reduce hexanal formation. Whereas levels of 150 ppm of rosemary essential oil were sufficient to inhibit lipid and protein oxidation, while higher concentrations (300 and 600 ppm) increase oxidation reactions in frankfurters formulated with Iberian pig. Therefore, the authors reported that the oxidative stability of the product depends on the concentration of the natural antioxidant used and on the components of the meat. In this sense, Pateiro, Barba, et al. (2018) claim that

some essential oils can act as pro-oxidants when added in high concentrations. Another study was carried out by applying rosemary essential oil to chicken fillets packed different conditions: air-packing and modified atmosphere (Kahraman et al., 2015). At concentrations of 0.2%, the rosemary essential oil inhibited the oxidation reaction, when in combinate with modified atmosphere packaging showed grater antioxidant activity and resulting in a reduction of lipid oxidation. All treatments added essential oil presented lower TBARS values than those no added. Although it had no effect on the growth rate of the bacteria tested, reduced the L* values and increased the values of a* in 5 and 7 days of storage at 4° C.

Armenteros et al. (2016) evaluated a blend of essential oils (garlic, cinnamon, clove and rosemary) and rose hip (*R. canina* L.) extract as natural antioxidant compared to a commercial antioxidant (citrate and sodium erythrobate, 1:1; w/w) in Iberian cooked ham via injected brine. In this study, after150 days of refrigerated storage, the mixture of essential oils (1 g/kg of meat) was more effective in controlling lipid oxidation on cooked ham than the other antioxidants studied. Regarding this, the authors affirmed that there was an abundant amount of antioxidant compounds in the spices studied, including carnosol and rosmarinic acid that was present in clove and rosemary. Whereas, for protein oxidation, rose hip and commercial antioxidant presented the lowest values after 150 days of storage. Rosehip was attributed to a high ability to prevent protein oxidation and carbonyl formation from proteins due to the high amount of ascorbic acid and other redox-active phenolic compounds. In this study, all antioxidants were effective against oxidation reactions compare to control (without antioxidants).

Spice essential oils were study as preservative in meat product by Sharma et al. (2017). Four different essential oils, clove oil (0.25%), holy basil oil (0.125%), cassia oil (0.25%) and thyme oil (0.125%), were incorporated into fresh chicken sausages packed in vacuum and stored at 18 ± 2 °C for 45 days. According to the authors, clove oil presented the lowest values of TBARS followed by cassia oil, however, all essential oils were effective against lipidic oxidation when compared to control. Regarding antimicrobial activity, cassia oil showed the lowest values in the total count of microorganisms, psychotropic and molds and yeasts. Sensorially, the essential oils added in chicken fresh sausage protected against discoloration during the storage.

The essential oil of *Satureja montana* L. was studied to evaluate its effects on TBARS and color in mortadella-type sausages with different concentrations of sodium nitrite (0, 100 and 200 $mg.kg^{-1}$) (Coutinho de Oliveira et al., 2012). S*atureja montana is* a plant native to the Mediterranean is widely used as a spice in food or as tea, and in its essential oil there is the

presence of compound such as thymol and carvacrol (terpenes) and some phenolic compounds, responsible for their biological proprietors. Although high concentrations of essential oil negatively impacted the color of the product (15.60 and 31.25 μg/g), it had its antioxidant activity confirmed, the use of lower concentrations of sodium nitrite was sufficient, as there was a synergistic effect of the essential oil (15.60 μl/g) with sodium nitrite (100 $mg.kg^{-1}$), presenting the lowest values of TBARS after 10 days of refrigerated storage.

Flowers, leaves and vegetable residues as naturals source of antioxidants applied in meat products

Extracts of leaves, flowers, and other plant residues rich in bioactive compounds are being studied as preservative in meat products due its antioxidant properties, being able to reduce or prevent oxidation reactions. Table 1-3 shows different works about the application of flowers, leaves and vegetable residues as naturals source of antioxidants in meat products.

Olive leaves, for example, are by-products of olive oil production and obtained in large quantities during tree pruning, harvesting and in olive oil industries. Olive leaves are rich in high-value bioactive compounds and there is scientific evidence that their polyphenols have antioxidant, anti-inflammatory, and hypoglycemic properties. Khemakhem et al. (2019) added olive leaf extract and hydrolyzed olive leaf extract at the concentration equivalent 200 mg gallic acid was added to each kilogram of salmon to produce salmon burger, as the authors claim to be sufficient for antioxidant activity. In this study, the added extracts increased the shelf life of the product, as they not only decreased the formation of volatile nitrogen bases and trimethylamine (indicators of deterioration in fish) but also reduced lipid oxidation during refrigerated storage (4 °C) when compared to the control.

Leaves from fruit trees are also being studied to obtain natural extracts to be applied in meat products as alternatives to synthetic antioxidants. For example, Lorenzo, Vargas, et al. (2018) evaluated the effect of pitanga leaf extract (250, 500 and 1000 $mg.kg^{-1}$) on lipid and protein oxidation in pork burger. The mains phenolic compounds found in the extract obtained are hydroxycinnamic acid (cinnamic and caffeic acids were the most abundant), tyrosol, alkylmethoxyphenols, hydroxycoumarins and hydroxyphenylpropenes, which are responsible for the high antioxidant activity in *in vitro* assay*,* resulting in a similar behavior to BHT (200 $mg.kg^{-1}$) in relation to lipid and protein oxidation. As expected, pitanga leaf extract and BHT maintained TBARS values below 0.5 mg MDA/kg during the 18 days of shelf life. The same

did not happen with the control (without antioxidant), which exceeded this value on the seventh day of refrigerated storage. In a different study, the addition of 250 $mg.kg^{-1}$of pitanga leaf extract in lamb burger was more efficient against lipid and protein from oxidation reactions than the other antioxidants used (BHT, 200 $mg.kg^{-1}$ and guarana seed extract, 250 $mg.kg^{-1}$) (de Carvalho et al., 2019).

To investigate the antioxidant activity of guava leaf extract (3000 to 6000 ppm), Tran et al. (2020) added it in fresh pork sausage kept under refrigeration and evaluated for 14 days. Control without antioxidant and treatment with BHT (200 ppm) were also elaborated. The extract added at concentrations of 4000, 5000 and 6000 ppm showed the lowest results for all oxidation parameters analyzed (conjugated dienes, peroxide value, acidic value and TBARS) that the control and its presented similar behavior to BHT. In addition, the guava leaf extract decreased discoloration during storage, highlighting the concentration of 4000 ppm as the most efficient in maintaining the characteristic red color desired by consumers.

The use of waste extracts from the food industry with ingredients in food is still quite challenging. According to Burri et al. (2020), little attention has been paid to fruit and vegetable processing residues and to the polyphenols present therein, however, applying appropriate extraction technologies, many by-products of these residues are more abundant in phenolic compounds than the fruits and vegetables themselves or processed products such as jellies and juices.

Extracts of thyme by-products, rich in bioactive compounds such as flavonoids, tannins, terpenoids among others, obtained through sustainable extraction by supercritical fluid were added to pork hamburger by Šojić et al. (2020). The authors obtained two extracts from the use of supercritical fluid extraction in two different conditions, and both had carvacrol, thymol and α-terpineol as the main terpenoids. The extracts were added at concentrations of 0.075 and 0.150 μL/g (four treatments) to patties and a control without addition of extract was also elaborated, which presented oxidation values above 0.5 mg MDA/kg from the second day of storage while the patties added thyme extract did not reach this value of TBARS even on the third day. In addition, the extracts avoided discoloration of the samples, confirming their use as an antioxidant in ground pork patties.

Three different industrial residues described as sources of phenolic compounds and with wide antioxidant capacity were studied by Munekata et al. (2017). Thus, extracts were obtained from the residues of beer production, chestnut leaves and peanut skin discarded during

Table 1-3 Flowers, leaves and vegetable residues as naturals source of antioxidants applied in meat products.

Source or active compound	Levels added	Sample	Storage conditions	Effect	Reference
Olive leaf extract (hydrolysate and not hydrolysate)	200 mg GAE/kg salmon	Salmon burger	Refrigeration storage condition (4 °C) for 16 days	Olive leaf extract (no hydrolysate) inhibit lipid oxidation better than the hydrolysate. Both reduced lipid oxidation compared to control and decreased the formation of volatile nitrogen bases and trimethylamine	Khemakhem et al. (2019)
Pitanga leaf extract	200, 500 and 1000 $mg.kg^{-1}$	Pork burger	Polystyrene trays sealed with polyethylene film – modified atmosphere (80% O_2 and 20% CO_2); Refrigerated storage (2 ± 1 °C) under light to simulate supermarket conditions for 18 days	Reduce the count of bacteria when compared to control and BHT at the end of storage. Improve color during the shelf-life (a*), decreasing the discoloration; all concentrations inhibited lipid and protein oxidation during the storage and shown similar behavior to BHT	Lorenzo et al. (2018)
	250 $mg.kg^{-1}$	Lamb burgers with fat replacement by chia oil emulsion	Package in modified atmosphere (80% O_2 and 20% CO_2) and storage at refrigeration (2 ± 1 °C) under light	Most efficient to reduce lipid and protein oxidation than BHT (200 $mg.kg^{-1}$) and guarana seed extract (250 $mg.kg^{-1}$); presented higher antioxidant activity than BHT and similar guarana seeds extract in 6 and 12 days of storage; avoided the formation of volatile compounds from lipid oxidation.	de Carvalho et al. (2019)
Guava leaf extract	3000 ppm, 4000 ppm, 5000 ppm and 6000 ppm on fat content basis	Fresh pork sausage	Packed in polyamide bags; Storage in the dark for 14 days at 4 °C	4000 to 6000 ppm results in the lowest results of oxidation parameters as conjugated dienes, peroxide value, acidic value and TBARS	Tran et al. (2020)

Source or active compound	Levels added	Sample	Storage conditions	Effect	Reference
Wild thyme by-products extract (supercritical fluid extraction in different conditions)	0.075 and 0.15 µL/g of extract	Ground pork patty	Packed in polypropylene trays and overwrapped with an oxygen-permeable polyvinyl chloride film and stored at 4 °C for 3 days.	TBARS values in pork patty with wild thyme by-products extract were lower than the control; protected proteins from oxidation; reduced discoloration during the storage time	Šojić et al. (2020)
Beer residue extract (BRE); Chestnut leaves extract; peanut skin extract	2000 $mg.kg^{-1}$	Spanish *salchichón* elaborated with encapsulated *n*-3 long chain fatty acids in konjac glucomannan matrix	-	BRE reduce protein oxidation and presented similar behavior to BHT; antioxidants were able to reduce the formation of aldehyde compounds and hexanal (volatiles compounds resulted from oxidation reactions)	Munekata et al. (2017)
Banana inflorescence extract	0.5, 1.0, 1.5 and 2.0 %	Raw pork sausage	Placed in polystyrene trays, covered with plastic wrap and stored for 28 days at 4 ± 1 °C	Inhibited lipid oxidation and the TBARs values do not reach the limits to sensorial detection at the end of storage for all concentrations of extract; even at 2%, the extract does not compromise the sensorial acceptance.	Rodrigues et al. (2020)

processing and such extracts were applied at the concentration of 2000 $mg.kg^{-1}$ in fermented product (Spanish *Salchichón)* prepared with encapsulated *n*-3 long chain fatty acids. There was no difference between treatments, including control, for the analysis of lipid oxidation by TBARS due to the protection of fatty acids generated by encapsulation. However, BHT and beer residue extract reduced protein oxidation when compared to the control and the antioxidants tested (synthetic and natural) was able to reduce the formation of aldehyde compounds.

Another residue studied as an antioxidant was banana inflorescences. In Brazil, bananas are the second most produced fruit, and more than 100 million tons of waste are generated from its crop, and it is important to use this waste in order to protect the environment (Schmidt et al., 2015, 2016). The inflorescence of bananas is the main residue of this cultivar and, after drying, becomes a by-product rich in potassium and fibers used to increase the nutritional quality of some foods, besides containing a high amount of antioxidant compounds (Padam et al., 2012). Lau et al. (2020), in a review on banana inflorescences and its application as an ingredient for functional foods state that this residue contains polyphenols such as phenolic acids (examples, gallic acid, p-hydroxylbenzoic acid, vanillic acid, caffeic acid and ferulic acid) and flavonoids such as catechin, epicatechin, quercetin and rutin, all considered to have antioxidant activity.

In this context, Rodrigues et al. (2020) investigated the antioxidant activity of the different parts of banana inflorescence and found that the extract obtained from male flowers presented the highest values for total phenolics and flavonoids. It also presented the highest antioxidant activity (FRAP) and lower CI_{50} (half of the maximum inhibitory concentration determined by the DPPH method). From the resulted, the extract of the male flower was added to raw sausage at concentrations of 0, 0.5, 1, 1.5 and 2% stored for 28 days at 4 °C. The authors reported that the concentrations used did not interfere with the sensory acceptance of the product and did not see an impact on its coloration, were effective in keeping the TBARS values lower than the control and, more importantly, kept them below the detection limit even after 28 days, which was exceeded by the control, according to the authors, in the fourth day of storage.

Other sources of natural antioxidants applied in meat products

Macroalgae and microalgae, present in abundance in the marine environment, can be important sources of natural antioxidants due to the presence of pigments in their cells such as chlorophylls and carotenoids, in addition to other compounds such as polyphenols, alkaloids,

tocopherol and terpenes (Agregán et al., 2018, 2019). *Haematococcus pluvialis* is rich in astaxanthin, a red-purple pigment that belongs to the carotenoids and considered one of the natural substances with the highest antioxidant capacity. In this context, *Haematococcus pluvial* extract containing 5% astaxanthin was added to the ground pork meat at concentrations of 0.15, 0.30 and 0.45 g/kg to evaluate its effect on the color and oxidative stability of this product stored under refrigeration by Pogorzelska et al. (2018). The extract studied showed high free radical inactivation capacity and, mainly, inhibited lipid oxidation at concentrations of 0.3 and 0.45 g/kg, increasing the oxidative stability of pig hamburgers and, in all concentrations, was effective in color stability, presenting the highest values of a*in this way favoring product acceptance. According to the authors, *Haematococcus pluvialis* seaweed extract can be applied to meat products as an antioxidant.

Also in pork patties, in this case formulated with *oleogels, Fucus vesiculosus* extract was added, a brown macroalgae rich in a phenolic compound (phlorotannins) known to have higher antioxidant capacity than commercial antioxidants (Agregán et al., 2019). The extract added in the proportion of 1000 $mg.kg^{-1}$ showed higher oxidative stability (lipid and protein) than the control and the other extract concentrations (250 and 500 $mg.kg^{-1}$) but was not as effective as the synthetic antioxidant BHT (200 $mg.kg^{-1}$). However, differences were not conserved between the results in sensory analysis, and the extract at 500 $mg.kg^{-1}$ promoted better scores for the odor attribute. For the authors, the benefits presented by *Fucus vesiculosus extract* were limited for later application in meat products, and one should study techniques to expand its antioxidant activity.

Final considerations

The use of extracts, essential oils or vegetable powders, industrial waste or different natural sources can extend the life of different meat products. The antioxidant capacity comes from bioactive compounds present in these vegetables as phenolic compounds such as phenolics acid, flavonoids, terpenes, terpenoids and other compounds that confer color such as curcuminoids, carotenoids and betacyanin's that can be found in barks, seeds, leaves, residues or by-products of vegetables, in addition to other non-plant sources, as seaweed. The main action to keep meat products with freshness characteristics for longer are their action against lipid oxidation, protein and against the discoloration of these products. Avoiding discoloration for longer is important to keep the product attractive throughout its useful life, because color

directly influences the consumer's purchase decision. Not least, the use of natural extracts in foods meets the new eating habits of consumers, who have increased their concern with health and are prioritizing consuming more natural foods with less preservatives.

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Capítulo 2

RED PITAYA EXTRACT AS NATURAL ANTIOXIDANT IN PORK PATTIES WITH TOTAL REPLACEMENT OF ANIMAL FAT

Elisa Rafaela Bonadio Bellucci, Paulo E.S. Munekata, Mirian Pateiro, José M. Lorenzo, Andrea Carla da Silva Barretto

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RED PITAYA EXTRACT AS NATURAL ANTIOXIDANT IN PORK PATTIES WITH TOTAL REPLACEMENT OF ANIMAL FAT

Elisa Rafaela Bonadio Bellucci[a], Paulo E.S. Munekata[b], Mirian Pateiro[b], José M. Lorenzo[b,c], Andrea Carla da Silva Barretto[a*]

[a] Department of Food Technology and Engineering, UNESP – São Paulo State University, Street Cristóvão Colombo, 2265, Zip Code 15054-000, São José do Rio Preto, SP, Brazil.

[b] Centro Tecnológico de la Carne de Galicia, Avda. Galicia nº 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain.

[c] Área de Tecnología de los Alimentos, Facultad de Ciencias de Ourense, Universidad de Vigo, 32004 Ourense, Spain

*Corresponding author:

Email: andrea.carla@unesp.br

Abstract – The antioxidant effects of red pitaya extract (PE) were evaluated in pork patties for 18 days at 2°C. The following treatments were prepared: control (CON, without antioxidant), sodium erythorbate (ERY, 500 mg.kg^{-1}), PE low dose (PEL, 250 mg.kg^{-1}), PE medium dose (PEM, 500 mg.kg^{-1}), and PE high dose (PEH, 1000 mg.kg^{-1}). No significant effect was observed on chemical composition and cooking loss with the addition of PE, while a significant effect was noticed in cohesiveness (P<0.05). The intense pink colour of PE enhanced the colour stability during storage (9.33, 7.92 and 7.69 *vs*. 6.77 for PEH, PEM and PEL *vs*. CON, respectively. TBARS (1.21 *vs*. 2.44 mg MDA/kg) and carbonyl values (5.45 *vs*. 6.87 nmol carbonyl/mg) of treated samples were lower than those observed in CON. Similar values were found between samples with PE and ERY. PE improved colour acceptance and the preference of pork patties. Therefore, PE is a very effective natural antioxidant by delaying colour and oxidative deterioration.

Keywords: Hylocereus polyrhizus, pulsed electric fields, oxidative stability, color enhancement

1. Introduction

Oxidative reactions reduce the quality of meat products (Fan et al., 2019; Ribeiro et al., 2019). The oxidation of lipids and proteins play a central role in this context and occur due to the exposure of unsaturated fatty acids (Domínguez et al., 2019) and other easily oxidized components to oxygen in processing steps such as grinding, slicing and cooking (Lorenzo et al., 2018; Zhang, Xiao, & Ahn, 2013). These oxidation processes reduce sensory (taste, colour and texture) and nutritional quality due to the degradation of fat-soluble vitamins and essential fatty acids, in addition to generating potentially harmful compounds (Fan et al., 2019; Ribeiro et al., 2019). Moreover, these reactions affect negatively the colour of both meat and meat products by favouring the formation of metmyoglobin that has a greyish brown colour (Pogorzelska, Godziszewska, Brodowska, & Wierzbicka, 2018). Consequently, these alterations cause a reduction in shelf life of meat products and affect the consumer´s acceptance resulting in the rejection of the product, since consumers often associate colour with freshness and meat quality (Agregán et al., 2019).

Additionally, some consumers do not associate animal fat and meat with positive health benefits (Agregán et al., 2019). In this sense, the health-related improvement of lipids in meat products are important to improve this perception among consumers and meet consumer's demands for healthy products (de Carvalho et al., 2019). Thus, animal fat replacement by other healthier sources of fat can be seen as a valuable strategy to obtain nutritionally enhanced meat products (Lorenzo, Munekata, Pateiro, Campagnol, & Domínguez, 2016; Moghtadaei, Soltanizadeh, & Goli, 2018) but oxidative reactions can occur due to the increase of unsaturated fatty acids (de Carvalho et al., 2019). The current trend is to avoid artificial food additives (Rubén Domínguez et al., 2020) and several studies have investigated the antioxidant effect of compounds extracted from natural sources as fruit extracts, food processing residues, seeds and leaves in meat products (Agregán et al., 2019; Cunha et al., 2018; de Carvalho et al., 2019; Fernandes, Trindade, Lorenzo, Munekata, & de Melo, 2016; Lorenzo, Munekata, et al., 2018; Lorenzo, Vargas, et al., 2018; Munekata et al., 2017; Pateiro et al., 2018).

Emerging technologies have been used to separate natural and high-added value compounds from vegetal sources (Chemat et al., 2020; Putnik et al., 2018). These alternative processing technologies allow to obtain higher quality compounds, since they operate at lower

temperatures and avoid organic solvents (Al Khawli et al., 2019). Pulsed electric fields (PEF) is a technique based on the application of high-voltage electrical pulses on treated samples during short periods, which results in the damage to the cell membrane, thus facilitating the extraction of entrapped bioactive compounds (Franco et al., 2020; Gómez et al., 2019).

Red pitaya or red dragon fruit (*Hylocereus monacanthus*) has attracted attention due to its red purple colour, and for having antioxidant activity associated with its polyphenol and betacyanin contents (Ramli, Ismail, & Rahmat, 2014; Wu et al., 2006). No studies have been found in the literature that assessed the influence of red pitaya extract on shelf life of meat products. This study was performed to assess the antioxidant activity of red pitaya extract and its effects on the physicochemical, oxidation stability (lipid and protein) and sensorial characteristics of pork patties with animal fat replacement during refrigerated storage.

2. Materials and Methods

2.1. Red pitaya extract (PE)

The pitaya fruit was obtained in the region of São José do Rio Preto, São Paulo, Brazil and the flesh was freeze-dried (Liotop L108, São Carlos, Brazil). The PEF extraction process (Energy Pulse Systems, Epulsus –PM1-10, Lisbon, Portugal) was carried out in the physicochemical laboratory of the Meat Technology Center of Galicia (Spain). Briefly, 5 g of pitaya freeze-dried pulp were diluted in 75 mL of water and then were agitated for 10 min at 50 rpm. PEF was applied under the following conditions: 5000 V, 10Hz with 200 pulses for 20 s. The extract was then agitated for 120 min at 150 rpm, centrifuged (10 min at 4500 rpm), filtered and freeze-dried (Labconco FreeZone 2.5 L, Kansas City, USA). All the extraction steps were carried out avoiding exposure to light.

2.2. Total phenolic content and antioxidant activity of the PE

2.2.1. Determination of Total Phenolic Content (TPC)

Total phenolic content was determined following the method described by Singleton, Orthofer & Lamuela-Raventós (1999) with some modifications. Appropriate dilutions of PE were prepared with water in test tubes (0.5 mL) and were mixed with diluted (1:10 with water) Folin-Ciocalteu reagent (2.5 mL) followed by 7.5% Na_2CO_3 solution (2 mL). After incubating

the test tubes at 50 °C for 15 min, the absorbance (760 nm) of the solutions was measured. The TPC 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 radical scavenging assay was carried out following the protocol described by Brand-Williams, Cuvelier & Berset (1995), with some modifications. 100 µL of samples were mixed with 3,900 µL of DPPH solution (60 µM in methanol). After incubating for 10 min at 37 ºC, the absorbance readings were performed at 515 nm. The results were expressed using Trolox as standard: mg Trolox equivalents (TE)/g sample.

2.2.3. Ferric Reducing Antioxidant Power (FRAP)

The protocol described by Benzie & Strain (1996), with some modifications, was used. The FRAP reagent was prepared in the same day of assay and was composed for the following solution on the ratio 10:1:1 (v:v:v): 0.3 M acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3$:$6H_2O$, respectively. Then, 30 µL of properly diluted samples were added to 900 µL of FRAP reagent and 90 µL of distilled water. After the incubation (37 °C for 20 min), the absorbance (593 nm) of solutions was measured. The results were expressed using $FeSO_4$ as standard: µmol Fe^{+2}/100g sample.

2.3. Manufacture of pork patties

The whole experiment was performed in duplicate on different days (separated two months in time) at the pilot plant of Meat Technology Center of Galicia (Ourense, Spain) using different meat raw material but the same ingredients and formulation. A total of 200 patties were produced: five pork patties samples for each treatment × five treatments (CON, ERY, PEL, PEM, PEH) × four times of sampling (0, 6, 12 and 18 days) × two different replications (blocks). Pork back fat was entirely replaced by tiger nut oil (obtained from the tubers of tiger nut) emulsion in the production of pork patties. Fat emulsion was prepared with water (56%), tiger nut oil (37.3%) and prosella (6.7%) [composed of jellifying agents (calcium sulphate and sodium alginate), wheat glucose syrup (7.4%), stabilizer (disodium diphosphate, added P2O5: 9.58%) and sodium ascorbate as antioxidant] (Prosella VG NF4, Colin Ingrédients, Mittelhausen, France) as described by de Carvalho et al. (2019) and kept refrigerated for 24

hours. 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, Pateiro, et al., 2020). The choice of this oil was based on previous studies, taking into account the nutritional benefits of meat products reformulated with this oil, and because not affect or even improve the sensory quality and the acceptability (Barros et al., 2020; Vargas-Ramella, Munekata, et al., 2020).

All formulations had the following ingredients: pork shoulder (*Triceps brachii*) ground in 8-mm-diameter mincing plate (750 g kg^{-1}), tiger nut oil emulsion ground in 6-mm-diameter mincing plate (150 g kg^{-1}), water (88 g kg^{-1}) and salt (12 g kg-^{1}). The ingredients were homogenized manually, and the meat mass was shaped into 0.1-m-diameter patties, 0.01 m height, with a patty-maker (A-2000, Gaser, Girona, Spain). After manufacture, patties 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 lower than 2 mL/m^2 bar/day; Viduca, Alicante, Spain) using a heat sealer (LARI3/Pn T-VG-R-SKIN, Ca.Ve.Co., Palazzolo, Italy). Samples were stored under fluorescent light during 18 days at 2 ± 1 °C, simulating the conditions from the supermarket. The trays were placed over metal shelving and receiving lux values in the range of 15–20 depending on the tray position (HT 306, Digital luxometer, Italy). The light source was conventional, so any wavelength or range was not filtered, in this case UV. Chemical composition, cooking loss, texture parameters and sensory analysis were analyzed on day 0, while pH, colour parameters, oxidative stability and *in vitro* antioxidant activity were evaluated and sampling to carry out the analysis was performed on days 0, 6, 12 and 18.

2.4. Chemical composition of pork patties

The recommend ISO standard methods were used to determine the ash (ISO 936, 1988), protein (ISO 937, 1978), and moisture (ISO 1442, 1997) of pork patties. The fat content was determined using the protocol of AOCS Official Procedure Am 5-04 (AOCS, 2004) using the Ankom^{XT10} (ANKOM Technology Corp., Macedon, NY, USA) equipment.

2.5. Colour parameters and pH of pork patties

A portable colorimeter (Konica Minolta CM-600d, Osaka, Japan) operating in the CIELAB space was used to determine the colour parameters. The measurements were obtained using illuminant D65 lighting (filtered from a pulsed xenon arc lamp) with an 8 mm aperture

size and 0° viewing angle geometry. The samples were exposed to air for 30 min to bloom before colour measurements. The total colour variation (ΔE*) was calculated to compare differences between CON and patties with PE during storage (Yudd & Wyszcki, 1975):

$$\Delta E^* = [(L^*_{6-18} - L^*_0) + (a^*_{6-1} - a^*_0) + (b^*_{6-18} - b^*_0)^2]^{1/2}$$

A digital portable pH-meter (model HI-99163, Hanna Instruments, Eibar, Spain) equipped with a penetration glass probe (model FC232D, Hanna Instruments, Eibar, Spain) was used to determine the pH of samples.

2.6. Cooking loss and texture profile analysis of pork patties

In order to evaluate the losses from cooking process, an automatic temperature control water bath (JP Selecta, Precisdg, Barcelona, Spain) with water at 80 °C was used to heat the packaged (80% vacuum) pork patties until the core temperature reach 70 °C. Type K thermocouples (Comark, PK23M, UK) connected to a data logger (Comark Dilligence EVG, N3014, UK) were used to control the temperature. Then, the samples were cooled until reaching room temperature (25 °C) outside the packaging and the percentage of cooking loss was calculated by the difference between the weights of raw and cooked samples and the results were expressed in percentage (%).

Texture profile analysis were carried out after cooking the samples as describe above for cooking loss assay and cooling them to room temperature (25 °C). Samples were cut into pieces of meat of 1 × 1 × 1 cm (height × width × length). Texture parameters (hardness, springiness, cohesiveness, gumminess and chewiness) were obtained using a texture analyser (TA-XTPlus, Stable Micro Systems, Godalming, UK) equipped with a cylindric probe (flat surface area of 19.85 cm^2). The equipment was calibrated prior to assay using a standard weight of 5 kg. The samples were compressed twice to 60% using 3.33 mm/s of crosshead speed to obtain the force-time curves. The software Texture Exponent 32 software (version 1.0.0.68, StableMicro Systems, Vienna Court, UK) was used to obtain the data.

2.7. Lipid oxidation and protein oxidation of pork patties

Lipid oxidation was determined through the methodology proposed by Vyncke (1975), which consists in the reaction between oxidation products with thiobarbituric acid to form compounds that can be measured spectrophotometrically at 532 nm. The 1,1,3,3

tetraethoxypropane (TEP) was used as standard for the standard curve and the results were expressed as mg of malonaldehyde (MDA)/kg of sample.

Protein oxidation was assessed using method developed by Oliver, Ahn, Moerman, Goldstein, & Stadtman (1987) with the adjustments proposed by Vuorela et al. (2005). Carbonyl and protein quantification were determined to assess protein oxidation. Briefly, 2.5 g of patties were homogenised with 20 mL of 0.6 M NaCl solution using an IKA T25 digital ultra-turrax (IKA®-Werke GmbH & Co. KG, Staufen, Germany). Then, 100 µL of homogenate were added to 1 mL of 10% trichloroacetic acid, agitated in vortex for 30 s and centrifuged at 5,000 g for 5 min. Two aliquots of 1 mL were collected from the supernatant: one aliquot was derivatized with 1 mL of 2 M HCl with 0.2% 2,4-dinitrophenyl hydrazine (DNPH) for carbonyl quantification whereas the other aliquot of supernatant was added to 1 mL of 2 M HCl for protein quantification and vortexed 30 s every 20 min for 1 h. Then, the solutions were centrifuged at 5,000 g for 5 min and supernatant carefully removed. The remaining pellets were washed thrice with 1 mL of ethanol/ethyl acetate (1:1) followed by a centrifugation at 10,000 g for 5 min after each washing. Afterwards, the pallets were homogenised with 1.5 mL of 6 M guanidine hydrochloride in 20 mM sodium phosphate buffer. The absorbance of solutions was measured at 370 and 280 nm to measure the carbonyl and protein content, respectively. The results were expressed using bovine serum albumin as standard: nmol of carbonyl/mg of protein.

2.8. In vitro antioxidant activity of pork patties

Following the protocol described by de Carvalho et al. (2019), the antioxidant capacity of pork patties was assessed *in vitro* using the DPPH radical scavenge method. Twenty mL of methanol were used to homogenize 1 g of lyophilized pork patties with an IKA T25 digital ultra-turrax (IKA®-Werke GmbH & Co. KG, Staufen, Germany). Then, the samples were centrifuged at 10,000 g for 10 min and filtered. The DPPH protocol was applied to the samples wherein Trolox was used as standard. A standard Trolox solution (0.3 g Trolox/L of methanol, prepared in darkness) was used to generate the standard curve with 7 known concentrations in the range of 0-1.2 mM. The samples were analysed using 100 µL of sample solution and 3.9 mL of 60 µM DPPH solution in methanol. The reaction was agitated in vortex and left for 10 min at 37 °C in a water bath. The inhibition percentage of absorbance (at 515 nm) was determined by the following equation:

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

The absorbance of the blank solution was determined by mixing 0.1 mL of methanol with 3.9 mL of 60 μM DPPH solution in methanol at time 0. The same step was applied to sample solutions and the corresponding inhibition percentage was used in the Trolox standard curve to express the results as μg Trolox/g.

2.9. Fatty acid profile of pork patties

The total fat was extracted following the protocol developed by Bligh & Dyer (1959) from 10 g of sample with modification proposed by Barros et al. (2020). After the extraction of fatty acids, the transesterification reaction was carried out following the procedure described by Domínguez, Crecente, Borrajo, Agregán & Lorenzo (2015) with some modifications. Briefly, 1 mL of toluene was used to dissolve 20 mg of fat prior to mixing with 2 mL of 0.5 N sodium methoxide solution in a test tube. This mixture was vortexed for 10 s and rested for 15 min at room temperature. Afterward, 4 mL of 10% of H_2SO_4 methanolic solution was added to the mixture that was vortexed for a few seconds. Then, the mixture was vortexed again for a few seconds after the addition of 2 mL of saturated sodium bicarbonate solution. Subsequently, the fatty acid methyl esters (FAMEs) were separated by adding 1 mL of hexane to the tube and vortexing for 10 s. Finally, the FAMEs were transferred to an appropriate GC vial.

Afterwards, the separation and quantification of FAMEs was carried out by gas chromatography (GC-Agilent 7890B, Agilent Technologies, Santa Clara, CA, USA) coupled to a flame ionization detector (FID) with an autosampler PAL RTC-120. The injection was carried out in split mode (1:50) with 1 μL, the injector was kept at 260 ºC, and the total flow was set to 64.2 mL/min. The separation was carried in an SP-2560 fused silica capillary column (100 m, 0.25 mm inner diameter (i.d.), 0.25-μm film thickness; Supelco Inc., Bellefonte, PA, USA). The selected carrier gas was helium with a flow rate of 1.2 mL/min. The pressure in the head of column was set to 42.135 psi. The chromatography conditions were set as follows: initial oven temperature of 140 ºC (held for 4 min), first ramp at 5 ºC/min to 190 ◦C, second ramp at 2 ºC/min to 210 ºC (held for 4 min), third ramp at 1 ºC/min to 220 ºC and fourth ramp at 3 ºC/min to a final temperature of 235 ºC (held for 7 min). The operational in FID was set as follows: temperature of 260 ºC, flow of H2 35 mL/min, air 350 mL/min, and make-up flow of 15 mL/min. The total time for chromatographic analysis was 50 min.

Authenticated standards (FAME Mix, 37 components, docosapentaenoic acid, *trans*-vaccenic acid, *cis*-vaccenic acid, and CLA) were used to identify the FAMEs by comparing the retention times. The content of each identified fatty acid was indicated as g/100 g of total identified fatty acids and the following groups were calculated: saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids, as well as *n*-6 and *n*-3 fatty acids, and their ratio (*n*-6/*n*-3).

2.10. Sensory analysis of raw and cooked pork patties

A total of 67 consumers from both genders with ages between 29 and 40 years old were selected from Ourense (Spain) to carry out the sensory analysis. Acceptance and preference tests were carried out at day zero in individual cabins with cooked pork patties. In the acceptance test, the panelist classified the samples using a hedonic scale (where 1 meant really dislike and 7 meant really like) for the following attributes: colour, odour, texture, taste and overall acceptance. The preference test was studied using an ordering test (UNE-ISO 8587: 2010), giving the score 1 to the least preferred and 5 to the most preferred. The pork patties were cooked until the core reached 70 °C in an oven (Rational CombiMaster® Plus CMP61, Landsberg am Lech, Germany). A 3-digit code was used to identify each sample and presented to panelists on plastic plates along with water and unsalted toasted bread to clean the palate and remove residual flavours (Macfie, Bratchell, Greenhoff, & Vallis, 1989). The same panelist carried out acceptance and preference tests.

2.10. Statistical analyses

The experiment was performed according to a randomized complete block design with two blocks (two runs separated two months in time). A total of 200 pork patties (five pork patties samples × five formulations (CON, ERY, PEL, PEM and PEH) × four sampling points (0, 6, 12 and 18 days) × two different processing batches) were analyzed for the different dependent variables.

For the statistical analysis, the normality and variance homogeneity of the data were previously evaluated (Shapiro-Wilk). The data were submitted to the two-way analysis of variance (ANOVA). The storage time and the treatment were considered as fixed variables, manufacture repetition was taken as random effect and the results of analysis (chemical

composition, colour parameter, pH, oxidation parameters, TPA, cooking loss, and fatty acid profile) were considered dependent variables. For the sensory analysis, the treatment was considered fixed effect and consumers as the random effect. The difference between the averages was assessed using the Tukey's *post hoc* test when ANOVA was significant (P<0.05). Particularly for the preference assay, the Friedman's test (significance level α <0.05) was used. The software SPSS (IBM SPSS Statistics, version 23) was used to perform the statistical analysis.

3. Results and discussion

3.1. Total phenolic content and antioxidant capacity of PE

TPC of the PE was 268.13 ± 6.17 mg of GAE/100 g. This result was lower than those found by Fidrianny, Ilham, & Hartati (2017), who reported values between 0.82 and 4.56 g GAE/100 g for extracts obtained from different sections of red pitaya (*Hylocereus costaricensis*) applying different polarity solvents (ethanol, n-hexane and ethyl acetate). In addition, the same study showed values between 0.82 and 3.75 g GAE/100 g in the pitaya flesh. These differences can be due to the extraction solvent (water was used in the present study). According to Haminiuk, Plata-Oviedo, de Mattos, Carpes, & Branco (2014), the extraction yield was influenced by solvent characteristics, affirming that less polar solvents were more efficient than water in the extraction of TPC. Nevertheless, Cunha et al. (2018) reported a similar outcome wherein ethanol was used as solvent (275 mg GAE/100 g pitaya peel extract). However, the results of this trial were higher than the data found by Wu et al. (2006) and Manihuruk, Suryati, & Arief (2017), who reported values of 42.4 mg GAE/100 g in flesh red pitaya and 31.12 mg GAE/100 g in red dragon fruit peel extracts, respectively.

The antioxidant activity was measured using FRAP, which is used to determine ferric reducing antioxidant power, and DPPH methods, which indicates the potential to reduce other compounds and scavenge free radicals (Tenore, Novellino, & Basile, 2012). FRAP assay revealed values of 825.40 μmol Fe^{+2}/100 g. The FRAP values obtained in present study were higher than those found by Ramli et al. (2014), who noticed FRAP values of 620 and 609.17 μmol Fe^{+2}/g dry extract for red dragon fruit (red pitaya) using ultrasound and conventional extraction, respectively. Moreover, Fu et al. (2011) found lower values (124 μmol $Fe^{+}2$/100 g) than those obtained in the present study. These variations are probably due to the differences in red pitaya variety, country of origin, planting, harvesting, and extraction conditions. Regarding

DPPH assay, Tenore et al. (2012) reported similar values in pitaya flesh (229 *vs*. 166.4 mg Trolox/100 g) to those found in the present study.

3.2. Chemical composition of pork patties

Similar values (P>0.05) were observed for moisture, protein, fat and ash content among treatments, showing that neither PE nor sodium erythorbate affected the composition of the pork patties (Table 2-1). The results were obtained by Agregán et al. (2019) were similar to those obtained in the present study. The authors indicated that the macroalgae *Fucus vesiculosus* extract with antioxidant activity did not affect the chemical composition of pork patties. Similarly, de Carvalho et al. (2019) did not observe significant differences among lamb patties produced with the incorporation of guarana (*Paullinia cupana*) seed and pitanga (*Eugenia uniflora L*.) leaf extracts. Both works were developed with fat replaced by healthy oils.

Table 2-1. Proximate composition, cooking loss and texture parameters of pork patties.

Parameters	Treatments					SEM	Sig.
	CON	ERY	PEL	PEM	PEH		
Proximate composition (%)							
Moisture	71.45	71.34	70.97	70.52	71.35	0.180	n.s.
Protein	15.28	15.29	15.12	15.44	15.63	0.181	n.s.
Fat	9.37	9.69	9.96	9.84	9.25	0.153	n.s.
Ash	2.57	2.59	2.52	2.55	2.59	0.010	n.s.
Cooking loss (%)	22.53	24.02	22.11	23.69	22.82	0.288	n.s.
Texture parameters							
Hardness (N)	128.32	136.65	127.36	139.55	132.73	2.41	n.s.
Springiness (mm)	0.78	0.77	0.76	0.76	0.75	0.005	n.s.
Cohesiveness	0.59^{b}	0.58^{ab}	0.56^{a}	0.57^{ab}	0.55^{a}	0.004	*
Gumminess (N)	75.55	78.77	72.57	81.29	74.00	1.49	n.s.
Chewiness (N·mm)	58.69	60.48	56.08	62.93	57.07	1.28	n.s.

SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; * P<0.05. Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with erythorbate at 500 mg kg^{-1}; PEM: patties prepared with red pitaya extract at 250 mg kg^{-1}; PEH: patties prepared with red pitaya extract at 500 mg kg^{-1}; PEL: patties prepared with red pitaya extract at 1000 mg kg^{-1}.

3.3. Cooking loss and texture profile analysis of pork patties

The incorporation of PE did not affect the cooking loss of the pork patties, showing values from 22.11 to 24.02 % for PEL and ERY treatments, respectively (Table 2-1). These values contrast with those found in beef burgers where tiger nut oil emulsion was also used as replacer of animal fat (Barros et al., 2020). These authors obtained mean values of 13.27% in the burgers where tiger nut oil emulsion entirely replaced beef fat.

The texture analysis revealed non-significant (P>0.05) effect was obtained among treatments (Table 2-1), except for cohesiveness. Therefore, PE had little effect on texture parameters. Regarding cohesiveness, CON samples displayed the highest values (P<0.05) in comparison to PE samples (0.59 *vs.* 0.55-0.57). Conversely, higher values (cohesiveness of 0.62) were reported for beef burgers produced with tiger nut oil (Barros et al., 2020).

3.4. pH and colour parameters of pork patties

The pH of pork patties during refrigerated storage is shown in Table 2-2. ERY treatment did not show significant difference over the storage time (P>0.05), while the pH of PEL, PEM and PEH increased on day 6 (from 5.59, 5.57 and 5.49 to 5.69, 5.63 and 5.63, respectively), followed by a reduction on day 12 (5.58, 5.50 and 5.45, respectively) (P<0.05). At the end of storage, the small differences found between treatments allow to affirm that pH of pork patties were not influenced by the addition of PE. These results were similar to those found by Garrido, Auqui, Martí, & Linares (2011), who reported values between 5.4 and 5.5 during 6 days of refrigerated storage in pork patties with grape pomace extract and packed in aerobic conditions. However, Pateiro et al. (2018) obtained higher values than those indicated in the Table 2-2. The authors found pH values around 5.83 with guarana seed extract in pork patties as natural preservative to improve shelf life.

It can also be noticed that pH values varied (P<0.05) among treatments of pork patties throughout the storage period. In this regard, ERY treatment presented the highest pH values during the refrigerated storage (0, 6, 12 and 18 days). In addition, these pH values were significantly lower compared with those observed in PEH treatment. Baldin et al. (2016) reported that microencapsulated jaboticaba extract (MJE) reduced the pH values of fresh sausages during the refrigerated storage. The authors related this reduction to the presence of carbohydrates in MJE. In addition, Lorenzo, Sineiro, Amado, & Franco (2014) also noticed that

the incorporation of antioxidants from tea and grape reduced the pH values of pork patties in relation to control group throughout storage time.

Table 2-2. Evaluation of pH values of pork patties during refrigerated storage

Treatments	Storage time (days)				SEM	Sig
	0	6	12	18		
CON	5.61^{abB}	5.67^{bB}	5.55^{aAB}	5.61^{abAB}	0.018	*
ERY	5.61^{B}	5.70^{B}	5.59^{B}	5.62^{B}	0.021	n.s.
PEL	5.59^{abB}	5.69^{bB}	5.58^{aB}	5.58^{aAB}	0.017	*
PEM	5.57^{abB}	5.63^{cA}	5.50^{aAB}	5.58^{cbAB}	0.022	*
PEH	5.49^{aA}	5.63^{bA}	5.45^{aA}	5.51^{aA}	0.022	*
SEM	0.024	0.012	0.015	0.018		
Sig	*	*	*	*		

$^{a–c}$ Mean values in the same row (same treatment in different days) with different letters indicate significant difference (P<0.05); $^{A-B}$ Mean values in the same column (different treatment in the same day) with different letters indicate significant difference (P<0.05); SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; * P<0.05. Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with erythorbate at 500 mg kg^{-1}; PEL: patties prepared with red pitaya extract at 250 mg kg^{-1}; PEM: patties prepared with red pitaya extract at 500 mg kg^{-1}; PEH: patties prepared with red pitaya extract at 1000 mg kg^{-1}.

The colour of pork patties was affected by both treatment and storage time (P<0.05) (Table 2-3). On days 0 and 18 of storage, no significant differences for L* values were noticed among the treatments. However, CON treatment presented the highest L* values and was significantly (P<0.05) different from PEH on day 6 (57.20 *vs.* 52.51) and from PEL and PEH on day 12 (56.67 *vs.* 53.67 and 52.64, for CON *vs.* PEL and PEH, respectively). All the treatments demonstrated a trend of reduction of L* values from day 0 to day 6, which was followed by an increased up to day 18.

All treatments presented a decreasing trend during the refrigerated storage for a* values (Table 2-3). This outcome is in agreement with de Carvalho et al. (2019), who observed the same trend in reduction of a* values during the storage, since the highest a* values were found in lamb burgers prepared with 500 ppm of natural extracts (guarana seed and pitanga leaf extracts). The results of this assay indicated that PEH group presented the highest a* values (13.14, 11.84, 10.21 and 9.33 at 0, 6, 12 and 18 days, respectively), followed by PEM and PEL treatments in all periods analysed, showing that the PE increased redness in pork patties. In addition, the intense pink colour of PE could help colour stability in meat products, acting as a natural colour when added in amounts equal or greater than 0.1%. Similarly to that obtained in

the present study, Pogorzelska et al. (2018) added *Haematococcus pluvialis* extract (rich in astaxanthin) to raw ground pork meat and observed that high concentrations of the extract increased a* values, which can be explained by the intense red-purple colour of extract. However, meat no underwent discoloration over time, but redness increased for all treatments. In sensory analysis, they realized that the increase of redness by the addition of extract improved consumer's acceptance and increased purchase intention of raw ground pork meat.

Table 2-3. Colour parameters of pork patties during refrigerated storage.

Parameters	Days	Treatments					SEM	Sig.
		CON	ERY	PEL	PEM	PEH		
L*	0	59.89^{AB}	58.63^{B}	59.06^{B}	61.38^{B}	58.38^{B}	0.437	n.s.
	6	57.20^{bA}	55.21^{abA}	54.17^{abA}	54.78^{abA}	52.51^{aA}	0.397	*
	12	56.98^{cA}	55.76^{abcAB}	53.67^{abA}	54.44^{abcA}	52.64^{aA}	0.400	*
	18	62.84^{B}	57.18^{AB}	61.67^{B}	59.57^{B}	60.07^{B}	0.685	n.s.
	SEM	0.688	0.472	0.639	0.643	0.703		
	Sig.	*	*	*	*	*		
a*	0	10.77^{aB}	10.66^{aC}	11.51^{abC}	11.13^{aB}	13.14^{bC}	0.249	*
	6	10.10^{abB}	9.76^{aBC}	10.99^{bcBC}	10.88^{bcB}	11.84^{cBC}	0.251	*
	12	9.08^{abB}	8.66^{aAB}	9.70^{abAB}	9.97^{bB}	10.21^{bAB}	0.160	*
	18	6.77^{aA}	6.60^{aA}	7.69^{abA}	7.92^{abA}	9.33^{bA}	0.292	*
	SEM	0.326	0.324	0.316	0.282	0.333		
	Sig.	*	*	*	*	*		
b*	0	20.79^{bB}	20.11^{abB}	20.55^{bC}	19.39^{abB}	18.47^{aC}	0.230	*
	6	18.91^{bA}	18.51^{bAB}	17.93^{abAB}	17.81^{abA}	16.84^{aAB}	0.197	*
	12	17.84^{bA}	18.23^{bA}	17.26^{bA}	17.35^{bA}	15.68^{aA}	0.187	*
	18	17.24^{A}	16.93^{A}	19.25^{BC}	17.20^{A}	17.99^{BC}	0.335	n.s.
	SEM	0.336	0.292	0.301	0.208	0.289		
	Sig.	*	*	*	*	*		
ΔE*	0-6	3.35^{a}	3.88^{a}	5.57^{b}	6.79^{cB}	6.23^{abB}	0.276	*
	0-12	4.48^{a}	3.97^{a}	6.57^{ab}	7.33^{bB}	7.02^{bB}	0.330	**
	0-18	6.11	5.36	4.81	4.29^{A}	4.20^{A}	0.398	n.s.
	SEM	0.513	0.401	0.289	0.428	0.395		
	Sig.	n.s.	n.s.	n.s.	*	*		

[a–b] Mean values in the same row (different treatment in the same day) with different letters indicate significant difference ($P<0.05$); [A-B] Mean values in the same column (same treatment in different days) with different letters indicate significant difference ($P<0.05$); SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; * $P<0.05$. Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with erythorbate at 500 mg kg^{-1}; PEL: patties prepared with red pitaya extract at 250 mg kg^{-1}; PEM: patties prepared with red pitaya extract at 500 mg kg^{-1}; PEH: patties prepared with red pitaya extract at 1000 mg kg^{-1}.

In general, b* values decreased for all the treatments studied as the storage progressed (Table 2-3). Until the twelfth day of storage, PE showed an effect on b* values when added at the highest concentration, resulting in a decrease (P<0.05) in b* values. Similarly, Sucu & Turp (2018) also reported that beetroot powder reduced b* values at 0 day in Turkish fermented beef sausage. Additionally, no significant differences (P>0.05) were found among the treatments on day 18 in this experiment.

ΔE* showed the total colour differences between CON and samples with PE along the storage time (Table 2-3). These colour differences could be due to the discoloration of the product because of oxidation processes during the storage. Samples with antioxidants possessed the lowest colour differences during storage (0.76, 1.48, 2.03, 2.50 and 2.76, for PEL, ERY, PEH, PEM and CON groups, respectively), which showed their greater oxidative stability compared to control samples. A related study indicated a similar trend in pork patties produced with natural antioxidants (Pateiro et al., 2018).

At the end of storage, the changes observed in the colour parameters were lower in PEM and PEH (4.20, 4.29 and 4.81 *vs*. 5.36 and 6.11, for PEH, PEM and PEL *vs*. ERY and CON, respectively). These results are in agreement with those found by other authors, who affirmed that the greater the amount of natural antioxidant added to the product, the lower the ΔE* value during storage (Estévez, Ventanas, & Cava, 2005). Except for the first sampling points, increasing the amount of PE was associated with lower ΔE*.

The values obtained were higher than those considered as noticeable (ΔE* higher than 2) (Francis & Clydesdale, 1975). However, the addition of PEM and PEL would allow these differences to be less noticeable by the consumer, confirming the positive effect of PE on colour stability.

3.5. Lipid and protein oxidation of pork patties

The evolution of lipid oxidation of pork patties is shown in Figure 2-1. Significant differences (P<0.05) were obtained during refrigerated storage among the treatments. TBARS values increased (P<0.05) over the storage time for all treatments. The values obtained up to day 6 are in agreement with the results reported by Pogorzelska et al. (2018), who observed values lower than 0.15 mg MDA/kg in raw grounded pork meat. At day 18 of storage, the highest value was obtained from CON (2.443 mg MDA/kg) and the lowest from ERY (0.901 mg MDA/kg).

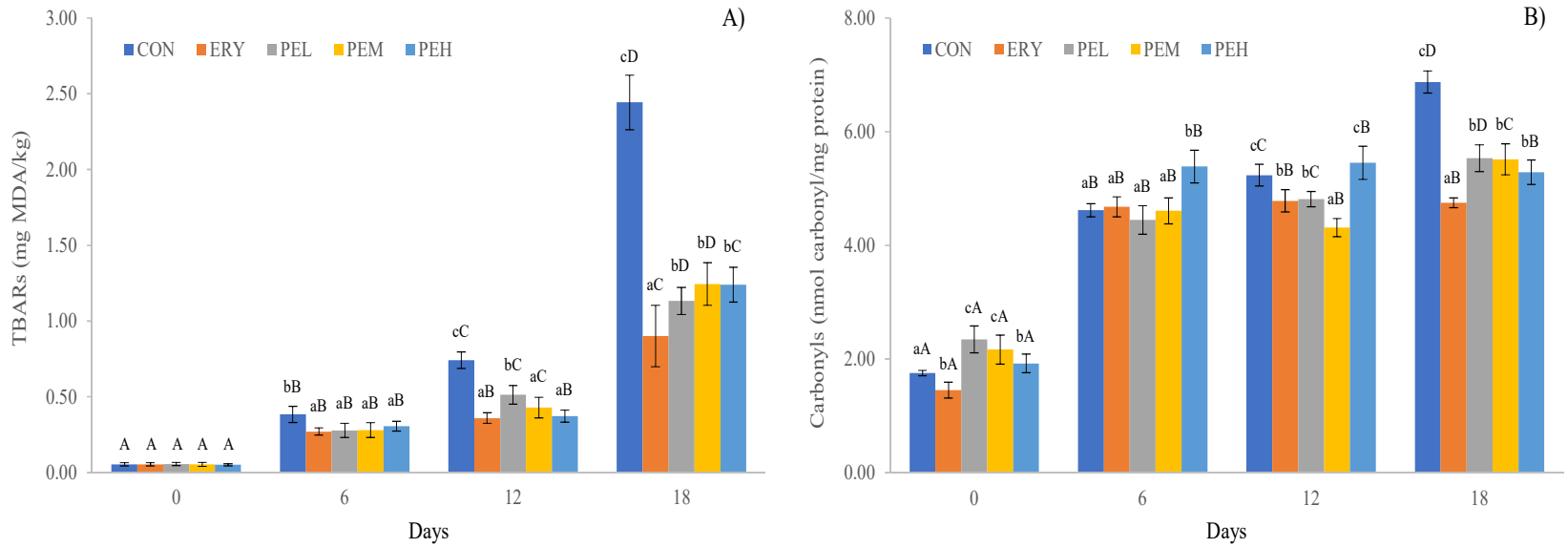

Figure 2-1. Evolution of TBARs values (A) and total carbonyl content (B) in pork patties during refrigerated storage.

[a–c] Mean values in the same day in the different treatment with different letters indicate significant difference ($P<0.05$); [A-D] Mean values in the same treatment in different day with different letters indicate significant difference ($P<0.05$); Errors bars corresponding to standard error. Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with erythorbate at 500 mg kg^{-1}; PEL: patties prepared with red pitaya extract at 250 mg kg^{-1}; PEM: patties prepared with red pitaya extract at 500 mg kg^{-1}; PEH: patties prepared with red pitaya extract at 1000 mg kg^{-1}.

The lower TBARS values obtained from pork patties produced with PE in comparison to CON samples, support the antioxidant potential of PE extract against lipid oxidation (1.13, 1.25 and 1.24 *vs*. 2.44 mg MDA/kg for PEL, PEM and PEH *vs*. CON treatments, respectively). This effect can be explained by the active compounds present in the extract such as polyphenols and betacyanin. Betacyanin is the most important pigment present in red pitaya and responsible for the characteristic colour of the fruit and for its antioxidant and radical scavenging activities (García-Cruz, Valle-Guadarrama, Salinas-Moreno, & Joaquín-Cruz, 2013; Ramli et al., 2014). A similar scenario was also observed with other natural extracts in patties (de Carvalho et al., 2019; Lorenzo et al., 2018; Pateiro et al., 2018). The oxidation values found for batches treated with PE are below the deterioration level, set at 2 mg MDA/kg (Campo et al., 2006).

It is relevant to mention that several studies suggest the possibility of applying natural extracts as substitutes for synthetic additives, since they are able to delay oxidative reactions in lipids in a similar or more effective way than their synthetic counterparts (Fernandes et al., 2017; Pateiro, Lorenzo, Amado, & Franco, 2014). This consideration is also supported by the results observed in the present study, especially between ERY and PEL samples (0.901 *vs*. 1.133 mg MDA/kg, respectively).

The quantification of the carbonyls formed by protein oxidation over refrigerated storage is shown in Fig. 1. There was a significant (P<0.05) increase of the carbonyl content throughout the shelf life of pork patties for all treatments. The most noticeable effect was observed at the end of storage (4.75, 5.29, 5.52 and 5.53 *vs*. 6.87 nmol carbonyl/mg protein, for ERY, PEH, PEM and PEL *vs*. CON treatments, respectively). These results are in line with those obtained by de Carvalho et al. (2019), and higher than those obtained in pork patties with other natural antioxidants (Lorenzo et al., 2018; Pateiro et al., 2018). Finally, no dose effect was observed on protein oxidation and treatments with antioxidants achieved inhibitions between 24% and 30% compared to CON treatment.

3.6. In vitro antioxidant activity of pork patties

In vitro antioxidant activity was evaluated through DPPH assay (Table 2-4). DPPH radical scavenging activity decreased significantly (P<0.05) in pork patties as storage time progressed up to 18 days. In this regard, de Carvalho et al. (2019) found the same decreasing trend in lamb burger with natural extracts obtained from guarana seed and pitanga leaf. In this study, ERY treatment presented the highest antioxidant activity at all evaluated sampling

points. However, at day 18, all treatments had similar antioxidant activity (P>0.05), but slightly higher in ERY and PEH samples (159.96 *vs*. 141.61 μg Trolox/g, respectively).

Table 2-4. In vitro antioxidant activity (μg Trolox/g) of pork patties during refrigerated storage.

Treatments	Storage time (days)				SEM	Sig.
	0	6	12	18		
CON	781.13^{cA}	254.96^{bA}	111.16^{aA}	91.43^{a}	45.979	*
ERY	1451.76^{cB}	615.34^{bC}	222.49^{aB}	159.96^{a}	84.990	*
PEL	766.84^{cA}	318.05^{bAB}	164.44^{aAB}	102.19^{a}	42.832	*
PEM	765.41^{cA}	365.39^{bB}	156.74^{aAB}	132.66^{a}	42.397	*
PEH	828.59^{cA}	407.71^{bB}	167.36^{aAB}	141.61^{a}	45.574	*
SEM	40.841	20.924	12.558	11.107		
Sig.	*	*	*	n.s		

$^{a–c}$ Mean values in the same row (same treatment in different days) with different letters indicate significant difference (P<0.05); $^{A-B}$ Mean values in the same column (different treatment in the same day) with different letters indicate significant difference (P<0.05); SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; * P<0.05. Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with erythorbate at 500 mg kg^{-1}; PEL: patties prepared with red pitaya extract at 250 mg kg^{-1}; PEM: patties prepared with red pitaya extract at 500 mg kg^{-1}; PEH: patties prepared with red pitaya extract at 1000 mg kg^{-1}.

The results of this trial contrast with those reported by Manihuruk et al. (2017), who noticed significant higher antioxidant activity in beef sausage produced with red dragon fruit peel extract in comparison to control (without antioxidant). Moreover, these authors also affirmed that the antioxidant capacity was proportional to concentration of extract (rich on polyphenols) used in the sausage. In this case, a dose-dependent effect was also observed, showing more activity at higher concentration. According to Mancini et al. (2015), *in vitro* antioxidant activity correlates with the oxidative stability. In other words: lipid oxidation increases as antioxidant activity of the pork patties decreases.

3.7. Fatty acid profile of pork patties

The influence of PE on fatty acid profile of pork patties is presented in Table 2-5. The MUFA represented more than 50% of the total fatty acids, ranging from 53.43% to 60.04 %, followed by SFA (between 27.14% and 29.03%). The fact of samples presented low SFA and

high MUFA could be related to the complete substitution of animal fat by the emulsion with tiger nut oil. A previous study with tiger nut oil emulsion indicated that this animal fat replacer contained low levels of SFA (around 21%), which was mainly composed of C16:0 (14%) and C18:0 (5.6%) (Barros et al., 2020). Moreover, the authors also indicated that tiger nut oil was mainly composed of C18:1*n*-9 and C18:2*n*-6 (67.2 and 10.2%, respectively). PEH and PEM treatments exhibited the highest values for MUFA, while CON batch presented the highest amount for SFA. Moreover, PUFA varied from 12.94% to 14.55%, with significantly (P<0.05) higher values in CON, PEL and ERY treatments.

Table 2-5. Fatty acid profile of pork patties (g/100 g of total fatty acids)

Fatty acids	Treatments					SEM	Sig.
	CON	ERY	PEL	PEM	PEH		
C14:0	0.63	0.54	0.56	0.54	0.53	0.012	n.s.
C16:0	16.75^{b}	16.21^{a}	16.38^{a}	16.39^{ab}	16.31^{a}	0.047	*
C16:1*n*-7	1.26^{ab}	1.19^{a}	1.23^{ab}	1.29^{bc}	1.34^{c}	0.011	*
C17:1*n*-7	0.18	0.17	0.17	0.17	0.16	0.008	n.s.
C18:0	9.41^{b}	9.15^{a}	9.15^{a}	9.19^{ab}	8.98^{a}	0.033	*
9t-C18:1	0.10	0.09	0.09	0.09	0.09	0.003	n.s.
11t-C18:1	0.22	0.16	0.15	0.16	0.13	0.018	n.s.
C18:1*n*-9	53.02^{a}	54.47^{bc}	53.85^{ab}	54.62^{bc}	55.48^{c}	0.177	*
C18:1*n*-7	2.02^{a}	2.01^{a}	2.05^{ab}	2.15^{bc}	2.21^{c}	0.015	*
C18:2*n*-6	12.71^{c}	12.38^{bc}	12.63^{c}	11.80^{ab}	11.27^{a}	0.107	*
C18:3*n*-3	0.49	0.42	0.49^{A}	0.41	0.39	0.012	n.s.
C20:0	0.53	0.57	0.55	0.56	0.57	0.007	n.s.
C20:1*n*-9	0.55	0.52	0.54	0.55	0.54	0.004	n.s.
C20:2*n*-6	0.35^{b}	0.33^{ab}	0.34^{b}	0.32^{ab}	0.29^{a}	0.005	*
C20:3*n*-6	0.12	0.11	0.11	0.10	0.10	0.002	n.s.
C20:4*n*-6	0.53	0.57	0.55	0.54	0.55	0.005	n.s.
SFA	28.02^{b}	27.14^{a}	27.30^{a}	27.33^{a}	27.01^{a}	0.078	*
MUFA	57.43^{a}	58.71^{b}	58.20^{ab}	59.13^{bc}	60.04^{c}	0.164	*
PUFA	14.55^{c}	14.15^{bc}	14.50^{c}	13.53^{ab}	12.94^{a}	0.124	*
n-3	0.69^{ab}	0.62^{ab}	0.71^{b}	0.62^{ab}	0.59^{a}	0.013	*
n-6	13.72^{c}	13.39^{bc}	13.64^{c}	12.78^{ab}	12.22^{a}	0.113	*
n-6/*n*-3	20.29	21.81	19.32	20.93	21.25	0.300	n.s.

$^{a–c}$ Mean values in the same row with different letters indicate significant difference (P<0.05); SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; * P<0.05. Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with erythorbate at 500 mg kg^{-1}; PEL: patties prepared with red pitaya extract at 250 mg kg^{-1}; PEM: patties prepared with red pitaya extract at 500 mg kg^{-1}; PEH: patties prepared with red pitaya extract at 1000 mg kg^{-1}.

Individually, it was observed that oleic acid appeared in greater quantity among the fatty acids (between 53.02% and 55.48%). PEH exhibited the highest value of oleic acid, and consequently, the highest MUFA content. Palmitic and stearic acids were the predominant SFA (16.21%-18.36% and 8.79%-9.41%, respectively). Moreover, linoleic acid was the main PUFA (10.43%-12.71%). In this regard, according with Barros et al. (2020), total replacement of animal fat by tiger nut oil gave rise to a meat product with low concentrations of SFA and high concentrations of unsaturated fatty acids. Therefore, this results in a healthier meat product since this type of product is usually made with significant amounts of pork fat. Animal fats, rich in SFA, are correlated with cardiovascular diseases, and some SFA such as myristic (C14: 0), palmitic (C16: 0) and steric acids (C18: 0) are associated with negative changes in the blood lipid profile (Ye, Cao, Li, Xu, & Liu, 2020). Moreover, it is also an important source of cholesterol, so its substitution by vegetable oils allows to reduce SFA with no associated cholesterol levels (Domínguez, Agregán, Gonçalves, & Lorenzo, 2016).

To evaluate the nutritional quality of the lipids, the ratio *n*-6/*n*-3 was determined (Table 2-5). The values obtained for this ratio were higher than the maximum limit recommended by FAO (2010), which must be less than 4. In this regard, Barros et al. (2020) also found values of *n*-6/*n*-3 ratio above of 4 (maximum limit recommended) in beef burger produced with tiger nut oil. Conversely, de Carvalho et al. (2019) observed lower values in lamb burgers using chia oil emulsion as a substitute for animal fat.

3.8. Sensory analysis of pork patties

The influence of the addition of PE on sensory attributes of pork patties is shown in Table 2-6. Significant differences ($P<0.05$) were obtained for colour among the different treatments of cooked pork patties. The inclusion of PE improved the scores for this sensory attribute. This result is in agreement with the instrumental colour parameters, where higher a* values were obtained from patties containing PE. Colour is the main attribute evaluated by consumers when purchasing meat products (Deda, Bloukas, & Fista, 2007). Therefore, the inclusion of PE into meat products can improve colour and acceptance of these products.

For the other attributes, being odour, taste, texture and overall acceptance, the panelists scored them similarly across treatments. In general, the scores remained between 5.27 and 6.21, which are above the acceptability limit (score = 4). This result shows that the inclusion of PE had no effect on odour, taste, texture and overall acceptance of pork patties. For the preference

test, PEH was the most preferred treatment (P<0.05) and was similar to PEM, PEL and ERY treatments, while CON group was the least chosen. To sum up, PE can improve the pork patties acceptance.

Table 2-6. Acceptance scores and sum of orders according to the preference of consumers on day 0 of cooked pork patties

	CON	ERY	PEL	PEM	PEH	SEM	Sig.
Acceptance test							
Colour	5.55^{ab}	5.24^{a}	5.94^{abc}	6.15^{bc}	6.36^{c}	0.087	*
Odour	5.91	5.85	6.21	5.97	6.21	0.081	n.s.
Taste	5.33	5.45	5.73	5.45	5.73	0.107	n.s.
Texture	5.58	5.67	5.82	5.67	5.88	0.092	n.s.
Overall acceptance	5.18	5.27	5.64	5.45	5.70	0.113	n.s.
Preference test	78^{a}	94^{ab}	104^{ab}	105^{ab}	114^{b}		*

$^{a–c}$ Mean values in the same row with different letters indicate significant difference (P<0.05); SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; * P<0.05. Hedonic scale: 1= really disliked; 2= disliked; 3= disliked a little; 4= did not like nor dislike; 5= liked a little; 6= liked; 7= really liked. Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with erythorbate at 500 mg kg^{-1}; PEL: patties prepared with red pitaya extract at 250 mg kg^{-1}; PEM: patties prepared with red pitaya extract at 500 mg kg^{-1}; PEH: patties prepared with red pitaya extract at 1000 mg kg^{-1}.

4. Conclusion

Red pitaya extract was able to protect the lipids and proteins from oxidation in pork patties. PE could also be used as a natural colorant to improve the consumer acceptance in meat products in concentrations equal to or greater than 0.1%. Other methods of extractions should be studied to increase the antioxidant potential of red pitaya in meat products. The animal fat replacement by tiger nut oil emulsion resulted in a product with a better nutritional profile, despite the high values of *n*-6/*n*-3 ratio. Thus, red pitaya extract can be considered a promising natural ingredient with favourable properties to improve colour and sensorial acceptance of meat products.

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Capítulo 3

AÇAÍ EXTRACT POWDER AS NATURAL ANTIOXIDANT ON PORK PATTIES DURING THE REFRIGERATED STORAGE

Elisa Rafaela Bonadio Bellucci, João Marcos dos Santos, Larissa Tátero Carvalho, Taís Fernanda Borgonovi, José M. Lorenzo, Andrea Carla da Silva Barretto

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Elisa Rafaela Bonadio Bellucci[a], João Marcos dos Santos[a], Larissa Tátero Carvalho[a], Taís Fernanda Borgonovi[a], José M. Lorenzo[b,c], Andrea Carla da Silva Barretto[a*]

[a] Department of Food Technology and Engineering, UNESP – São Paulo State University, Street Cristóvão Colombo, 2265, Zip Code 15054-000, São José do Rio Preto, SP, Brazil.

[b] Centro Tecnológico de la Carne de Galicia, Avda. Galicia nº 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain.

[c] Área de Tecnología de los Alimentos, Facultad de Ciencias de Ourense, Universidad de Vigo, 32004 Ourense, Spain

*Corresponding author: andrea.carla@unesp.br

Abstract - The current trends among consumers are pushing for the use of natural antioxidants options. Açaí fruit is rich on polyphenolic components but no studies have been carried out to evaluate their effect in meat products. The objective was to investigate the effect of açaí extract on refrigerated pork patties quality. Five treatments were done: without antioxidant (CON), Sodium Erythorbate 500 mg.kg $^{-1}$ (ERY), Açaí Extract: 250 (AEL), 500 (AEM), 750 mg.kg $^{-1}$ (AEH). Açaí extract did not affect the proximate composition, pH and cooking parameters. The concentrations of açaí extract studied increased antioxidant activity and reduced lipid oxidation (0.379, 0.293, and 0.217 *vs*. 0.889 mg MDA.kg^{-1} for AEL, AEM, AEH *vs.* CON, respectively). However, only the AEL treatment did not affect the color parameters, showing the best option for the application on pork patties. Thus, açaí extract at 250 mg.kg^{-1} cans be used as a natural antioxidant replacing sodium erythorbate to preserve the quality of refrigerated pork patties.

Keywords: antioxidant activity, *Euterpe oleracea*, lipid oxidation, meat quality, natural extract

1. Introduction

The widely and massive consumption of meat products associated with the increasing world population and concerns about the impact on food components have generated important challenges for the food industry, especially in meat sector to produce healthier meat products (Barone et al., 2021). According to Teixeira & Rodrigues (2021), consumer perceptions in recognizing healthier meat products involve factors like physical and chemical composition, nutritional quality, sensory properties and new ingredients. In addition, consumers are adept at plant-based ingredients and recognize them as more natural (Barone et al., 2021).

The development of healthier meat products is a complex task that involves also the preservation of quality of the meat product. One of the main factors that influencing the quality of meat and meat products is oxidative deterioration (Munekata et al., 2020). Oxidative reactions cause discoloration and affect the flavor and aroma, which lead to consumer rejection. Other important effects are the production of potentially toxic compounds and reduction of nutritional quality (Amaral, Silva, & Lannes, 2018; Lorenzo, Trindade, Ahn, & Barba, 2019). The nutritional quality of meat products is a relevant factor, which can be reduced due to undesirable reactions that occur with lipid oxidation such as losses in vitamins, essential fatty acids and essential amino acids (Domínguez et al., 2019). In meat products like patties, the lipid oxidation can increase because the meat grinding process leads to the rupture of the membrane exposing phospholipids to oxygen, accelerating the development of oxidative rancidity, since one of the main factors for the development of lipid oxidation is the exposure to oxygen (Zamuz et al., 2018). The products of lipid oxidation favor the oxidation of heme-proteins, increasing their pro-oxidant activity, and also induce the oxidation of myoglobin, which alters its color from bright cherry-red color to brownish tones (Tomasevic, Djekic, Font-i-Furnols, Terjung, & Lorenzo, 2021).

Several studies in recent years showed the protective effect of plant-based antioxidants in meat products (Alirezalu et al., 2020). In this regard, Turgut, Işıkçı, & Soyer (2017) evaluated the antioxidant effect of pomegranate peel extract in relation to the oxidation of lipids and proteins in beef meatballs during the frozen storage (-18 ± 1 ° C) and concluded that the addition of 0.5 and 1% of pomegranate peel extract in meatballs decreased the oxidation of lipids and proteins and increased the sensory scores, demonstrating that the extract delayed the lipid and protein oxidations. In addition, Reis et al. (2017) investigated the microencapsulated extract of propolis co-product on the stability of burgers during storage at -15 °C and confirmed its efficiency against the lipid oxidation. Moreover, Ferreira et al. (2017) incorporated the extracts

from acorns in chicken patties and found that the extract reduced the deterioration of color and texture and delayed the lipid and protein oxidation in patties over the refrigerated storage.

Munekata et al. (2020) noticed that the main bioactive components of plants that can be used in the meat industry are polyphenols (such as anthocyanins, tannins and flavonols) and essential oils (mainly composed of terpenes). Also, the antioxidant and antimicrobial mechanisms exerted by phenolic compounds justify their use as additives in the preservation of meat products (Munekata et al., 2021). In this context, Açaí (*Euterpe oleracea*), a native fruit from Amazon region, is widely known for its bioactive compounds (Carvalho et al., 2017), for its high antioxidant activity and for the great potential as a functional food or ingredient (Garzón, Narváez-Cuenca, Vincken, & Gruppen, 2017; Kang et al., 2010; Silva et al., 2019). The major polyphenolic components in açaí pulp include anthocyanins, proanthocyanidins, other flavonoids and lignans (Carvalho et al., 2017; Kang et al., 2011).

Several studies have investigated the antioxidant properties of açaí pulp (Garzón et al., 2017; Hogan et al., 2010; Kang et al., 2011; Paz et al., 2015) and its by-products (Brunschwig et al., 2016; Martins et al., 2020; Melo et al., 2021), which support the further investigation of açaí as potential natural antioxidant for its application in food products. Although, these studies provide important evidence to explore the natural antioxidants from açaí, the effect in a complex system involving components susceptible to oxidative degradation (such as lipids and myoglobin in meat products) remains poorly explored. Therefore, the objective of this work was to obtain an extract from the açaí pulp, characterize its antioxidant properties and evaluate the addition of açaí extract in pork patties on the physic-chemical properties and oxidative and color stability during the refrigerated storage.

2. Materials and Methods

2.1. Preparation of Açaí extract (AE) powder and determination of antioxidant activity

2.1.1. Preparation of AE

Açaí pulp was obtained from a producer located in São José do Rio Preto (São Paulo, Brazil) on the day of pulp extraction from the ripe açaí, showing deep purple color. The pulp was taken to the Department of Food Engineering and Science (UNESP, São José do Rio Preto - São Paulo, Brazil) where the extraction and experiment with the açaí extract was carried out. For the extraction, water was added to the pulp in the proportion of 1:1 and then was agitated for 30 min at 150 rpm (under protection from the light) and centrifuged (4500 rpm for 20 min).

The supernatant was filtered, frozen and freeze-dried. The resultant powder of açaí extract was used to total phenolic compound and antioxidant activity analyses and added to pork patties at three different levels.

2.1.2. Total phenolic compounds (TPC) and antioxidant activity of AE

The açaí extract powder was mixed in diluted methanol (1:3 in water) and the total phenolic compound test was carried out following the method described by Singleton, Orthofer, & Lamuela-Raventós (1999) with some modifications. The appropriate dilution of AE with water was made in test tubes (0.5 mL) and mixed with 2.5 mL of diluted reactive Folin-Ciocalteu (1:10 in water). After 2 min, 7.5% Na_2CO_3 solution (2 mL) was added, and the test tubes were incubated at 50 °C for 10 min and the absorbance (760 nm) of the solutions was measured. The standard curve was prepared with concentrations in the range of 0-100 mg of gallic acid/L and the TPC was expressed as mg of gallic acid equivalent (GAE)/g sample.

The DPPH assay test was carried out following the protocol described by Brand-Williams, Cuvelier, & Berset (1995), with some modifications. The 100 μL of AE were mixed with 3,900 μL of DPPH solution (60 μM in methanol). After incubation for 10 min at 37 ºC, absorbance (515 nm) was measured. The standard curve was elaborated using Trolox (0-1.2 mM) and the results were expressed as mg Trolox equivalents (TE)/g sample.

2.2. Manufacture of pork patties

The experiment was performed in the pilot plant of the Department of Food Engineering and Technology, Institute of Biosciences, Letters and Exact Sciences - UNESP, São José do Rio Preto, São Paulo, Brazil. A total of 180 pork patties was elaborated: four pork patties for each treatment x five treatments (CON, ERY, AEL, AEM and AEH) x three sampling points (0, 5 and 10 days) x three different replication (on different days with different raw materials and the same ingredients). The treatments were: CON (without antioxidant), Sodium Erythorbate (ERY, 500 $mg.kg^{-1}$), Açaí Extract at three levels: low (AEL, 250 $mg.kg^{-1}$), medium (AEM, 500 $mg.kg^{-1}$), and high (AEH, 750 $mg.kg^{-1}$).

All treatments were produced with: pork shoulder ground in 8-mm mincing plate (*Triceps brachii*) (780 g kg^{-1}), pork backfat ground in 8-mm mincing plate (150 g kg^{-1}), salt (15 g kg^{-1}) and textured soy protein (10 g kg^{-1}). The raw material meat was acquired in local market

in the city of São José do Rio Preto, Brazil contemplating the Federal Inspection Service 48 hours after slaughter. Water was added to the treatments in order to complete 1 kg. To produce the pork patties, the ingredients were homogenized manually for 10 min and the açaí extract was dissolved in water before the addition. The meat mass was shaped in a manual burger former (0.1 m diameter and 0.01 m height). The samples were packed in nylon-polyethylene bags, sealed without vacuum, stored at 2 ± 1 °C for 10 days in the dark and evaluated at 1, 5 and 10 days.

2.3. Proximate composition and pH

Proximate composition of pork patties was determined following the official method of the Association of Official Analytical Chemistry (AOAC, 2007) for moisture, protein and ash content. Fat content was determined following the method proposed by Bligh & Dyer (1959). The results were expressed in g/100 g. The pH values were measured directly on pork patties using a digital pH meter mPA210 (Tecnopon, São Paulo, Brazil) equipped with a penetration glasses electrode previously calibrated with two standard solutions (pH 4 and pH 7) at room temperature. In both proximate composition and pH assays, four determinations were made in each treatment.

2.4. Instrumental color parameters

Instrumental color parameters were measured on the surface of uncooked pork patties, avoiding areas with a lot of fat. The blooming time was 20 min before analysis, while the samples were exposed to air. ColorFlex45/0 colorimeter (Hunterlab, Reston, United States) was used and black glasses and white tiles were utilized for calibration. Samples were measured using illuminant with 0.75-inch aperture size and 10° viewing angle geometry. The CIELab system of color specification was used, and the parameters obtained were L* (lightness), a* (redness) and b* (yellowness). Chroma (Equation 1) and hue angle (Equation 2) were calculated using the parameters a* and b*. Three determinations were made in each sampling patty and four samples were used for each treatment.

$$C^* = \sqrt{(a^2 + b^2)} \qquad (1)$$

$$h° = \arctan\left(\frac{b^*}{a^*}\right) \qquad (2)$$

2.5. Cooking proprieties

In order to measure the loss from cooking process, cooking loss test was carried out and raw pork patties from each treatment were weighed, placed on a baking sheet, covered with aluminum foil and roasted in an electric oven at 100 °C until the internal temperature reached 71 °C. Afterward, the samples were cooled until they reached room temperature and were weighed again. For shrinkage, the diameter was measured three times in each pork patty before and after cooking.

Cooking loss (CL) was calculated as follows:

$$CL\ (\%) = \frac{(Initial\ weight\text{-}Final\ weight)}{Initial\ weight} \times 100$$

The diameter reduction (DR) was calculated as follows:

$$DR\ (\%) = \frac{(Initial\ diameter\text{-}Final\ diameter)}{Initial\ diameter} \times 100$$

2.6. Lipid oxidation

Lipid oxidation (TBARS) was determined by reacting the oxidation products with thiobarbituric acid (TBA) to form compounds that can be measured spectrophotometrically at 532 nm, according to the method described by Vyncke (1975). Briefly, sample (one pork hamburger for each treatment) was completely homogenized, and 5 g were homogenized with 25 mL of 7.5% trichloroacetic acid solution for 2 min with an Ultra-Turrax (IKA®-Werke GmbH & Co. KG, Staufen, Germany). The homogenate was centrifuged (Rotina 420R, Hettich Zentrifugen, Germany) at 3500 rpm for 10 min. The supernatant was obtained after filtration and 5 mL reacted with 5 mL of 0.02 M TBA solution and heated in a water bath (96 °C for 40 min). Açaí pigments are soluble in water, so they can be present in supernatant before the addition of thiobarbituric acid solution. Thus, TBARS determination were performed with minor modifications as described by Bellucci et al. (2021) to avoid pink color interfere. Prommachart et al. (2020) modified this method when studied the black rice water extract in beef patties to avoid the interference of color. The 1,1,3,3 tetraethoxypropane (TEP) was used as standard and the results were expressed as mg of malonaldehyde (MDA)/kg of sample.

2.7. Radical scavenging activity assay (in vitro)

The pork patties extract was obtained as described by Prommachart et al. (2020), with modifications. Briefly, 3 g of grounded pork patty was homogenized with methanol (10 mL) using an IKA T18 digital ultra-turrax (IKA®-Werke GmbH & Co. KG, Staufen, Germany) (13,000 rpm) for 2 min and the homogenate was centrifuged at 10,000 x g at 4 °C for 10 min (Rotina 420R, Hettich Zentrifugen, Germany) to collect a clear supernatant. The DPPH (Radical scavenging activity assay) was determined as described by Bellucci, Munekata, Pateiro, Lorenzo, & da Silva Barretto (2021), where the samples were analyzed using 100 µL of sample solution and 3.9 mL of 60 µM DPPH solution in methanol. The reaction was agitated in vortex and left for 10 min at 37 °C in a water bath. The inhibition percentage of absorbance (at 515 nm) was determined by the following equation:

$$\%\ Inhibition = \frac{Abs\ (blank) - Abs\ (sample)}{Abs\ (blank)}\ x\ 100$$

The blank was made from the mixture of 100 µL of methanol and 3.9 mL of 60 µM DPPH solution in methanol.

2.8. Statistical analyses

Five treatments (CON, ERY, AEL, AEM, AEH) of pork patties were manufactured in triplicate (n = 3), and analyzed during the refrigerated storage (at 1, 5 and 10 days). For the statistical analysis of the results, analysis of variance (ANOVA) was carried out using the General Linear Model (GLM). The time of storage and the treatment were considered to be fixed effects, while manufacturing repetition was a random effect. Statistical analyses were carried out using the software STATISTICA version 7 (StatSoft, Inc, 2004).

3. Results and discussion

3.1. Total phenolic compounds (TPC) and antioxidant activity of AE

According to Paz et al. (2015), TPC (determined by Folin–Ciocalteu method) does not measure the amounts of phenolic compounds, but instead measures the chemical reducing capacity of compounds present in the extract relative to gallic acid. So, the TPC found in this study for AE was of 612.54 ± 17.64 mg of GAE/g of extract. This is considerably higher than the value (6.07 ± 2.17 mg GAE/g fresh weight) previously reported by Garzón, Narváez-

Cuenca, Vincken, & Gruppen (2017) in Colombian açaí. Lower values also were observed by Paz et al. (2015), who noticed value of 1808 mg of GAE/100 g of açaí extract and by Hogan et al. (2010) who studied an anthocyanin-rich extract from açaí and reported value of 312 mg of GAE/g of extract. In this study, the level of TPC can be considered high, because it was higher than 2,500 mg of GAE/100 g (Vasco, Ruales, & Kamal-Eldin, 2008).

The antioxidant activity, measured using DPPH assay, is a technique based on the reduction of the DPPH radical in the presence of a hydrogen-donating antioxidant. The DPPH values found in this study were of 47.57 ± 0.83 μmol TE/g of AE (or 1190 mg TE/100 g of AE). This result is similar to that found by Paz et al. (2015) (1574 ± 101 mg TE/100 g) and lower that those reported by Hogan et al. (2010) who noticed values of 1208 ± 130 μmoles TE/g of anthocyanin-rich extract from açaí. Vasco et al. (2008) analyzed the antioxidant activity of major fruits from Ecuador and found values between 0.3 and 76 μmol TE/g sample (fresh weight) for DPPH assay.

3.2. Proximate composition and pH of pork patties

As expected, no differences were observed for the moisture, protein, fat and ash contents (*P>0.05*) among the pork patties (Table 3-1).

Table 3-1 Proximate composition of pork patties with sodium erythorbate and different levels of açai extract (AE) during refrigerated storage.

Parameters	CON	ERY	AEL	AEM	AEH	SEM	Sig.
Moisture	63.82	61.83	66.16	64.83	66.15	0.828	n.s.
Protein	19.13	20.47	20.39	19.83	20.33	0.372	n.s.
Fat	11.94	12.66	10.69	11.02	10.57	0.324	n.s.
Ash	2.25	2.25	2.36	2.37	2.46	0.033	n.s.

SEM: standard error of the mean; Sig.: Significance; n.s. Not significant (P<0.05). Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with sodium erythorbate at 500 mg kg^{-1}; AEM: patties prepared with açai extract at 250 mg kg^{-1}; AEH: patties prepared with açai extract at 500 mg kg^{-1}; AEL: patties prepared with açai extract at 750 mg kg^{-1}.

These results showed that neither açaí extract nor sodium erythorbate interfered in the proximate composition of the pork patties. These findings agree with data reported by Bellucci et al. (2021) who did not find significant differences in chemical composition of pork patties

elaborated with the addition of a natural extract (from pitaya) and sodium erythorbate. In addition, de Carvalho et al. (2020) also observed that turmeric extract did not affect the proximate composition of fresh lamb sausages.

As for the proximate composition, the pH values (Table 3-2) did not display significant differences among treatments (P> 0.05), showing that the açaí extract and sodium erythorbate did not affect the pH values of refrigerated pork patties. In addition, in this study, it was also observed that the pH values also did not differ between sampling times in all analyzed treatments (P> 0.05). Likewise, Prommachart et al. (2020) also found no effect on pH values when black rice water extract was added to beef patties.

Table 3-2 Evaluation of pH values of pork patties with sodium erythorbate and different levels of açai extract (AE) during refrigerated storage.

Days	CON	ERY	AEL	AEM	AEH	SEM	Sig
1	5.74	5.75	5.88	5.73	5.78	0.029	n.s.
5	5.68	5.78	5.76	5.75	5.73	0.019	n.s.
10	5.69	5.70	5.77	5.69	5.75	0.031	n.s.
SEM	0.058	0.024	0.022	0.019	0.008		
Sig	n.s.	n.s.	n.s.	n.s.	n.s.		

SEM: standard error of the mean; Sig.: Significance; n.s.: Not significant (P<0.05). Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with sodium erythorbate at 500 mg kg^{-1}; AEM: patties prepared with açai extract at 250 mg kg^{-1}; AEH: patties prepared with açai extract at 500 mg kg^{-1}; AEL: patties prepared with açai extract at 750 mg kg^{-1}.

In this study, the pH values ranged from 5.69 to 5.88 and were similar to those found by Pateiro et al. (2018) in pork patties with addition of guarana seeds extract (from 5.67 to 5.87), also during the cold storage. In addition, the authors reported differences among treatments from the eleventh day. However, slightly lower pH values (from 5.45 to 5.70) were reported by Bellucci et al. (2021) in pork patties with pitaya extract until 18 days under refrigerated conditions, but this difference can be justified by the differences in packaging conditions and, also, can be inherent to the meat used.

3.3. Cooking parameters

The results of cooking loss and shrinkage tests of pork patties with sodium erythorbate and different concentrations of açaí extract during the refrigerated storage are shown in Table 3-3. The cooking loss of pork patties varied between 22.99% and 29.12% and did not show significant differences (*P>0.05*) among treatments and storage times. Cooking loss promotes the release of matrix fluids that carry water, water-soluble nutrients and compounds responsible for flavor and aroma, as well as color-forming pigments. Thus, the addition of sodium erythorbate and different concentrations of açaí extract in pork patties did not influence the texture, the sensory and nutritional characteristics of the product, as well as the cooking yield (Pedroso & Demiate, 2008).

Table 3-3 Cooking loss and shrinkage of pork patties with sodium erythorbate and different levels of açai extract (AE) during refrigerated storage.

Parameters	CON	ERY	AEL	AEM	AEH	SEM	Sig.
Cooking loss (%)							
1	25.152	24.720	23.089	24.662	24.307	0.513	n.s.
5	25.276	24.910	22.992	23.873	23.805	0.695	n.s.
10	26.910	26.510	26.596	27.649	29.122	0.462	n.s.
SEM	0.738	0.896	0.679	0.735	0.907		
Sig	n.s.	n.s.	n.s.	n.s.	n.s.		
Shrinkage (%)							
1	16.645	14.704	16.876	15.952	15.484	0.299	n.s.
5	15.158	14.755	16.385	16.160	15.759	0.364	n.s.
10	16.948	16.865	16.921	18.204	17.077	0.348	n.s.
SEM	0.388	0.534	0.353	0.446	0.436		
Sig	n.s.	n.s.	n.s.	n.s.	n.s.		

SEM: standard error of the mean; Sig.: Significance; n.s.: Not significant (P<0.05). Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with sodium erythorbate at 500 mg kg^{-1}; AEM: patties prepared with açai extract at 250 mg kg^{-1}; AEH: patties prepared with açai extract at 500 mg kg^{-1}; AEL: patties prepared with açai extract at 750 mg kg^{-1}.

The percentage of shrinkage of pork patties varied from 14.70% to 18.20% and did not show significant differences (*P> 0.05*) among treatments over the whole display. Avoiding shrinkage is a main goal to preserve the quality standards of patties, due to the potential negative responses from consumers that resemble such changes with an excess of added water (Sánchez-Zapata, Pérez-Alvarez, & Fernández-López, 2012). The diameter reduction is the consequence

of the denaturation of proteins, evaporated water and loss of fat, therefore, the addition of sodium erythorbate and different concentrations of açaí extract did not influence the diameter reduction and yield.

3.4. Instrumental color parameters

The color of pork patties was affected by both treatment and storage time ($P<0.05$) (Table 3-4). A trend was observed where the luminosity (L*) increased over the storage time for all treatments. The CON and ERY samples did not differ in any sampling point, however; patties prepared with açaí extract showed lower values of L*. Prommachart et al. (2020) reported that black rice extract decreased lightness in beef patties due to the presence of anthocyanin. Similar results were reported by Lee et al. (2016) in cooked pork patties prepared with brown soybean extract (rich source of various phenolic and anthocyanin compounds).

Regarding a* values, all treatments presented a decreasing trend during the refrigerated storage (Table 3-4). This outcome is in agreement with Lorenzo et al. (2018), who also observed the same trend in a* values in pork burgers prepared with pitanga leaf extract. This result is due to the oxidation of lipids and meat pigments occur simultaneously and one increases the other. Myoglobin and hemoglobin are the main heme-proteins in animal tissue and for the development of rancidity, these heme-proteins are oxidized, compromising the color of the meat (Domínguez et al., 2019), and, in this study, it is possible to compare the reduction of a* values with the increase of lipid oxidation during the storage period for all treatments. At 1 and 5 days of storage, AEH presented the lower a* value ($P<0.05$), followed by AEM treatment. Up to 10 days of storage, the lower a* value was found in CON (5.24). It means that açaí extract affected a* value, preserving the redness in comparison with CON group (without antioxidant).

The AE addition also affected b* values ($P<0.05$) in all sampling points (Table 3-4). The decrease in b* value in all treatments was directly proportional to the increase in AE concentration, where AEH treatment showed the lowest b* values, followed by AEM and AEL batches, respectively. The same trend was observed by Prommachart et al. (2020) in beef patties with black rice extract, where the higher concentration of the extract presented the lowest b* value. The authors also affirmed that these samples presented a greater bluish tinge. For both b* and Chroma values, CON, ERY and AEL treatments presented a decrease in b* values over the storage time, while AEH and AEM treatments did not show difference among the sampling points.

Table 3-4 Color parameters of pork patties with erythorbate and different levels of açai extract (AE) during refrigerated storage.

Parameters	Days	CON	ERY	AEL	AEM	AEH	SEM	Sig.
L*	1	55.32^{Ba}	54.83^{ab}	52.34^{Bbc}	49.98^{Bcd}	47.42^{Bd}	0.427	*
	5	57.18^{Ba}	56.50^{a}	53.86^{ABab}	53.20^{Ab}	50.56^{Ab}	0.437	*
	10	61.58^{Aa}	57.02^{b}	55.99^{Abc}	52.88^{ABc}	49.95^{ABd}	0.649	*
	SEM	0.637	0.476	0.402	0.430	0.468		
	Sig.	*	n.s.	*	*	*		
a*	1	9.74^{Aa}	9.91^{Aa}	9.96^{Aa}	8.49^{Aab}	7.15^{Ab}	0.210	*
	5	7.83^{Ba}	8.46^{Ba}	7.98^{Ba}	6.43^{Bb}	6.04^{Bb}	0.183	*
	10	5.24^{Cb}	6.83^{Ca}	6.74^{Bab}	5.88^{Bab}	5.86^{Bab}	0.176	*
	SEM	0.365	0.280	0.283	0.230	0.183		
	Sig.	*	*	*	*	*		
b*	1	16.15^{Aa}	16.16^{Aa}	14.98^{Aa}	12.33^{b}	10.66^{c}	0.243	*
	5	15.33^{ABa}	15.14^{Ba}	13.72^{ABb}	11.90^{c}	11.10^{c}	0.196	*
	10	15.19^{Ba}	14.40^{Ba}	13.52^{Bab}	11.75^{b}	10.43^{c}	0.296	*
	SEM	0.161	0.168	0.189	0.188	0.183		
	Sig.	*	*	*	n.s.	n.s.		
C*	1	18.32^{Aa}	18.38^{Aa}	17.43^{Aa}	14.04^{b}	11.98^{c}	0.368	*
	5	17.07^{ABa}	17.38^{Aa}	15.01^{Bb}	13.11^{c}	12.27^{c}	0.295	*
	10	16.15^{Ba}	15.96^{Ba}	16.99^{Aa}	13.19^{b}	12.01^{b}	0.303	*
	SEM	0.257	0.259	0.334	0.242	0.233		
	Sig.	*	*	*	n.s.	n.s.		
h°	1	60.97^{Bab}	61.26^{Ba}	56.62^{Bab}	56.38^{Bb}	56.62^{Bb}	0.593	*
	5	67.02^{Aa}	62.88^{ABb}	61.96^{Ab}	63.62^{Aab}	61.54^{Ab}	0.481	*
	10	71.02^{Aa}	64.57^{Ab}	60.80^{Ab}	62.42^{Ab}	60.84^{Ab}	0.723	*
	SEM	1.127	0.566	0.704	0.619	0.641		
	Sig.	*	*	*	*	*		

$^{a–b}$ Mean values in the same row (different treatment in the same day) with different letters indicate significant difference ($P<0.05$); $^{A-B}$ Mean values in the same column (same treatment in different days) with different letters indicate significant difference ($P<0.05$); SEM: standard error of the mean; Sig.: Significance; n.s.: Not significant; * $P<0.05$. Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with sodium erythorbate at 500 mg kg^{-1}; AEM: patties prepared with açai extract at 250 mg kg^{-1}; AEH: patties prepared with açai extract at 500 mg kg^{-1}; AEL: patties prepared with açai extract at 750 mg kg^{-1}.

Hue angle presented lower values at 1 day followed by an increase after 5 days (*P<0.05*), showing not significant differences on hue angle at 10 days. Prommachart et al. (2020) associated the hue with the discoloration and the authors correlated the increase in hue angle with more discoloration. Therefore, in our study, it was observed that AE treatments

showed the lower hue angle after 10 days of storage, whereas the higher angle hue was observed in the control group (without antioxidant) followed by ERY treatment. So, AE was able to reduce discoloration in pork patties during the refrigerated storage. In this regard, Prommachart et al. (2020) also found that 1.2% black rice extract was able to reduce discoloration in beef patties. Concerning color parameters, it is important to highlight that AEL treatment did not differ from sodium erythorbate treatment. Figure 3-1 presents the color results obtained instrumentally and it is possible to observe that after cooking AEL and ERY samples remained similar to each other. Therefore, açaí extract at 250 mg.kg^{-1} can be used in pork patties without affecting its color and consequently its acceptance by the consumer because color is the first impression by the consumer for any meat product and is usually the basis for choosing or rejecting the product (Tomasevic et al., 2019).

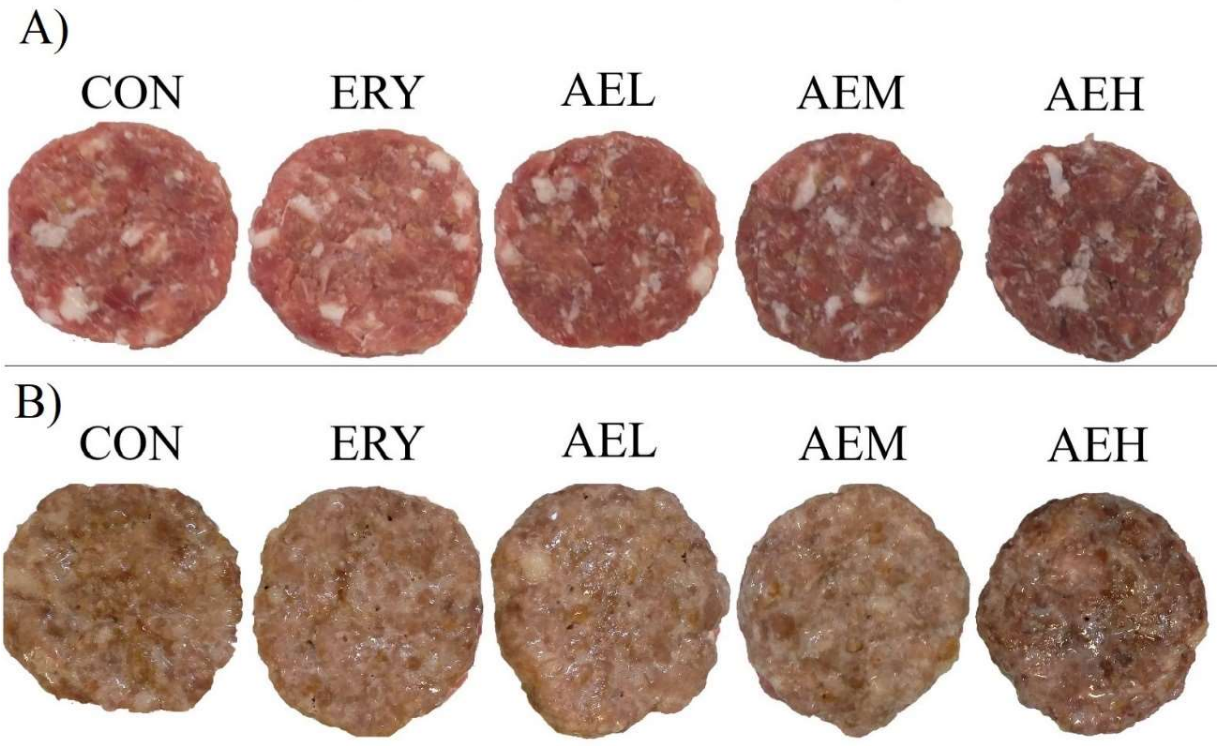

Figure 3-1 Photographs of the A) raw pork patties, B) cooked pork patties.

Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with erythorbate at 500 mg kg^{-1}; AEM: patties prepared with açai extract at 250 mg kg^{-1}; AEH: patties prepared with açai extract at 500 mg kg^{-1}; AEL: patties prepared with açai extract at 750 mg kg^{-1}.

3.5. Lipid oxidation

Lipid oxidation in pork patties is expressed through TBARs values and significant differences were observed among treatments ($P<0.05$) (Table 3-5). Sodium erythorbate and AE (all the levels studied) were effective to delay the lipid oxidation, where the samples prepared with antioxidants presented the lowest TBARS values in all sampling points. Consequently, control treatment displayed higher lipid oxidation values than other treatments throughout the storage period (0.448, 0.677 and 0.889 mg MDA/kg of sample for 1, 5 and 10 days, respectively). According to Campo et al. (2006), oxidation is the main factor of deterioration in meat and the limits of perception vary according to the sensitivity and experience of the panelist and can differ according to the origin of the meat (bovine, pork, etc.). It is also important to highlight that only CON treatment reached the limit values for the sensory perception of rancidity (0.5 to 1.0 mg MDA/kg of sample) for pork meat (Tarladgis, Watts, Younathan, & Dugan, 1960).

Table 3-5 Evolution of TBARs values of pork patties with sodium erythorbate and different levels of açai extract (AE) during refrigerated storage.

Day	TBARs (mg of MDA/g sample)						
	CON	ERY	AEL	AEM	AEH	SEM	Sig
1	0.448^{Ba}	0.100^{Bb}	0.238^{Bb}	0.170^{Bb}	0.151^{Bb}	0.026	*
5	0.677^{ABa}	0.242^{Ab}	$0.326A^{Bb}$	0.276^{Ab}	0.206^{Ab}	0.035	*
10	0.889^{Aa}	0.240^{Ab}	0.379^{Ab}	0.293^{Ab}	0.217^{Ab}	0.049	*
SEM	0.058	0.024	0.022	0.019	0.008		
Sig	*	*	*	*	*		

$^{a–b}$ Mean values in the same row (different treatment in the same day) with different letters indicate significant difference ($P<0.05$); $^{A-B}$ Mean values in the same column (same treatment in different days) with different letters indicate significant difference ($P<0.05$); SEM: standard error of the mean; Sig.: Significance; n.s.: Not significant; * $P<0.05$. Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with sodium erythorbate at 500 mg kg^{-1}; AEM: patties prepared with açai extract at 250 mg kg^{-1}; AEH: patties prepared with açai extract at 500 mg kg^{-1}; AEL: patties prepared with açai extract at 750 mg kg^{-1}.

The TBARS values obtained from lower concentrations of açaí extract (250 $mg.kg^{-1}$) did not differ from ERY samples in all sampling points (1, 5 and 10 days) and this result supports the antioxidant potential of AE extract against the lipid oxidation. This antioxidant effect can be explained by the active compounds present in the extract, as according to Garzón et al. (2017), the high antioxidant activity from açaí extract is linked with the presence of

flavonoids such as orientin, homoorientin, vitexin, luteolin, chrysoeriol, quercetin, and dihydrokaempferol. Flavonoids are able to scavenge or quench oxygen free radicals or excited oxygen species. In addition, its action mechanism can inhibit oxidative enzymes to generate these reactive oxygen species (Kang et al., 2011; Paz et al., 2015).

Similarly, Bellucci et al. (2021) also found an effective extract against the lipid oxidation from pitaya in pork patties. In addition, the authors observed an increase in TBARS values over the storage time for all treatments and this behavior was also observed in this study, where the TBARs values increased from 1 to 10 days of storage ($P<0.05$). A similar behavior was observed by others authors (Fernandes et al., 2018; Lorenzo et al., 2013; Lorenzo et al., 2014; Pateiro et al., 2018) who added natural extracts into meat products.

3.6. In vitro antioxidant activity of pork patties

In vitro antioxidant activity of pork patties was measured through DPPH assay and the results are shown in Figure 3-2. Among all evaluated sampling points, ERY treatments presented the highest antioxidant activity. In this regard, Bellucci et al. (2021) also found the same trend since patties with açaí extract showing higher antioxidant activity compared with the control group (without antioxidant). This finding also agrees with data reported by Prommachart et al. (2020), who found lower values of antioxidant activity in control without antioxidant compared with treatments of beef patties added of black rice extract.

4. Conclusion

The addition of açaí extract (250, 500 and 750 $mg.kg^{-1}$) improved the antioxidant status of pork patties but caused changes on color parameters at medium and high levels. The low level addition (250 $mg.kg^{-1}$) showed less changes on color parameters than the other levels of açaí extract studied. This treatment also displayed a similar protective effect against the lipid oxidation that the samples elaborated with sodium erythorbate. Therefore, at 250 $mg.kg^{-1}$, açaí extract can be used as a natural antioxidant to replace sodium erythorbate to preserve the quality and extend the shelf-life of chilled pork patties.

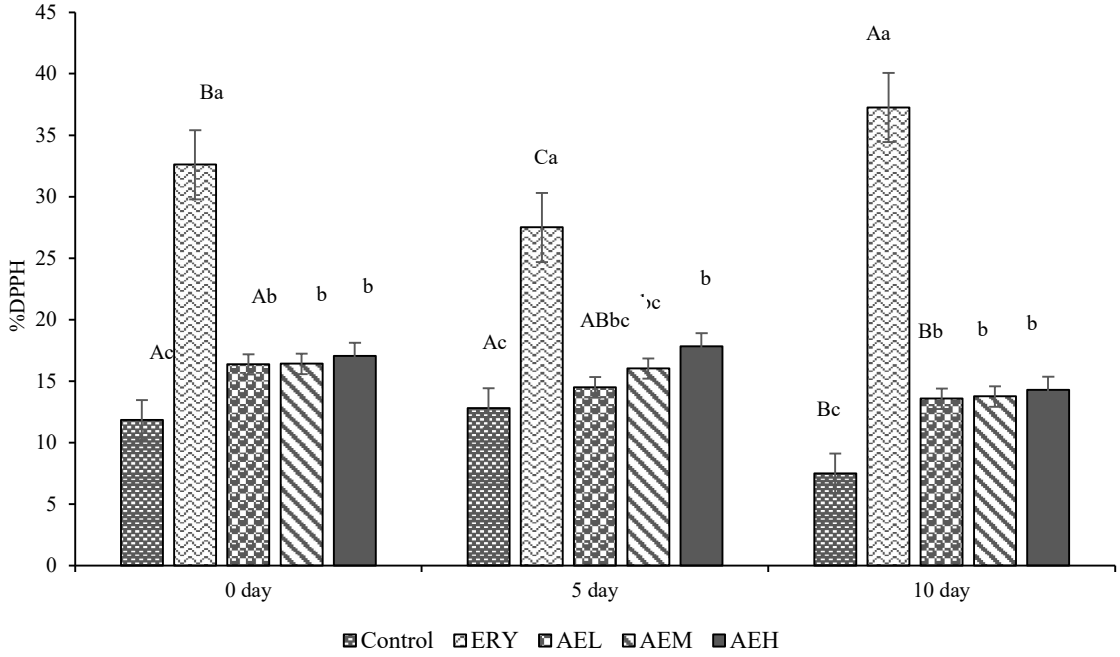

Figure 3-2 Antioxidant activity (%DPPH) of pork patties with erythorbate and different levels of açai extract (AE) during 10 days of refrigerate storage.

[a–c] Mean values in the same day in the different treatment with different letters indicate significant difference ($P<0.05$); [A-C] Mean values in the same treatment in different day with different letters indicate significant difference ($P<0.05$); Errors bars corresponding to standard error Treatments: CON: patties prepared without antioxidant; ERY: patties prepared with erythorbate at 500 mg kg^{-1}; AEM: patties prepared with açai extract at 250 mg kg^{-1}; AEH: patties prepared with açai extract at 500 mg kg^{-1}; AEL: patties prepared with açai extract at 750 mg kg^{-1}.

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Capítulo 4

APPLICATION OF AÇAÍ EXTRACT POWDER AS NATURAL ANTIOXIDANT IN ITALIAN-TYPE SALAMI UNDER RIPENING CONDITIONS: EVALUATION ON PHYSICOCHEMICAL AND MICROBIOLOGICAL PROPERTIES

Elisa Rafaela Bonadio Bellucci, Larissa Tátero Carvalho, João Marcos do Santos, José M. Lorenzo, Andrea Carla da Silva Barretto

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Elisa Rafaela Bonadio Bellucci[a], Larissa Tátero Carvalho[a], João Marcos do Santos[a], José M. Lorenzo[b,c], Andrea Carla da Silva Barretto[a]

[a] Department of Food Technology and Engineering, UNESP – São Paulo State University, Street Cristóvão Colombo, 2265, Zip Code 15054-000, São José do Rio Preto, SP, Brazil.

[b] Centro Tecnológico de la Carne de Galicia, Avda. Galicia nº 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain.

[c] Universidade de Vigo, Area de Tecnología de los Alimentos, Facultad de Ciencias de Ourense, 32004 Ourense, Spain

*Corresponding author:

Email: andrea.carla@unesp.br

Abstract - Consumers have been concerned about the consumption of chemical additives, increasing the demand for products made with natural ingredients and the development of clean label meat products by industry. The aim of this study was to evaluate the addition of açai extract as a natural antioxidant in Italian-type salami on the physical-chemical properties and lactic bacteria count, in addition to oxidative and color stability during processing. Five treatments were performed: without antioxidant (CON), sodium erythrobate 500 $mg.kg^{-1}$ (ERY), açaí extract with 250, 500 and 750 $mg.kg^{-1}$ (EA250, EA500 and EA750, respectively). Results were obtained for centesimal composition, pH, color, weight loss, water activity, lactic bacteria, texture profile and TBARS values. When included in Italian-type salamis, açaí extract did not affect centesimal composition and hardness parameters, however, it helped reduce water activity and pH values, increased the lactic acid bacteria population in EA500 and EA750. The TBARS values obtained

from the added treatments of açaí extract did not differ from the ERY treatment at all processing times and this result evidence the antioxidant potential of açaí extract against lipid oxidation. Regarding color parameters, only EA250 treatment was not significantly affected. Therefore, the addition of acai extract (500 and 750 $mg.kg^{-1}$) can be used as a natural antioxidant as a substitute for sodium erythrobate to improve quality and extend shelf life of Italian-type salamis.

Key words: dry-fermented sausage, natural additive, açaí extract, healthy meat products.

1. Introduction

Consumers are increasingly making buying food a decision-making process, evaluating their labels more critically, equating brands and, most importantly, analyzing the list of ingredients. In the United States and some European countries, the existence of food additives is one of the major concerns of consumers and, due to globalization, are also growing in emerging markets, encouraging the implementation of more natural ingredient solutions, the called *clean label*. Recently, clean-labeled foods claimed to replace chemical or artificial additives with natural ingredients, as well as preserving high quality from less invasive technologies (ASIOLI et al., 2017). According to Sucu & Turp (2018), the risk associated with chemical additives make consumers to preferer natural additives in meat products.

Due to the potential toxicological effects caused by synthetic food additives and in order to meet consumer demands, the meat industry currently shows a strong interest in the use of natural ingredients in place of synthetic ingredients (NIKMARAM et al., 2018). Numerous research has been conducted in recent years aimed at using spice extracts, fruits and vegetable residues as natural antioxidants to conserve meat products and make them healthier (BASANTA et al., 2018; BELLUCCI et al., 2022; ECHEGARAY et al., 2018; ŠOJIĆ et al., 2020; TRAN et al., 2020; WÓJCIAK; DOLATOWSKI, 2016).

Salami is a dry fermented sausage elaborated with raw meat and fatty tissues, added of fermentation cultures, salts, curing agent, sugars and spices. In general, processes stapes such as fermentation, drying and curing result in reduced pH value and

water activity, inhibition of pathogens, and they are responsible for formation of desire sensory properties as flavor, texture and color (BIS-SOUZA; PENNA; DA SILVA BARRETTO, 2020). The aim of this study was to evaluate the addition of a extract obtained from açai, a fruit natural from Amazon region and is widely known for having high antioxidant activity *in vitro* due its rich composition in phenolic compounds such as different anthocyanins, flavones, and phenolic acids (Carvalho et al., 2017; Gordon et al., 2012), as a natural antioxidant in Italian-type salami on the physical-chemical properties and lactic bacteria count, in addition to oxidative and color stability during processing.

2. Materials and Methods

2.1. Obtaining and characterization of açaí extract powder extract

The samples of açaí pulp used were acquired from a producer located in the District of Talhado, belonging to the city of São José do Rio Preto, São Paulo, Brazil, responsible for the production and pulping of açaí. The pulp was obtained at the producer's processing plant as follows: reception and weighing, sanitization, first selection and softening, rinsing, second selection and drainage, pulping, filtering, homogenization, filling and freezing until the moment of obtaining the açaí extract.

For extraction, water was added to the pulp at a ratio of 1: 1 and then was agitated for 30 min at 150 rpm (protected from light) and centrifuged (4500 rpm for 20 min). The supernatant was filtered, frozen and lyophilized. The açaí extract (AE) was characterized in previously study concerning total phenolics compounds (612.54 mg Gallic Acid Equivalent (GAE)/100g of açaí extract) and antioxidant capacity by DPPH assay (47.57 μmol Trolox Equivalent (TE)/g of açaí extract) (Bellucci et al., 2022).

2.2. Manufacturing of Italian-type salami added with açaí extract powder

The experiment was carried out in the pilot plant of the Department of Food Engineering and Technology, Institute of Biosciences, Letters and Exact Sciences - UNESP, São José do Rio Preto, São Paulo, Brazil. Five Italian-type salami formulations were prepared with different levels of açaí extract: CONT: control formulation without the addition of antioxidants, ERY: with the addition of 500 $mg.kg^{-1}$ of sodium erythorbate, EA250: with 250 $mg.kg^{-1}$ of açaí extract, EA500: with 500 $mg.kg^{-1}$ of açaí extract and, finally, EA750: with 750 $mg.kg^{-1}$ of açaí extract. The starter culture Bactoferm® T-SPX

(*Staphylococcus xylosus* + *Pediococcus pentosaceus*; Chr-Hansen, Hørsholm, Denmark) was used in all treatments and was dosed as recommended by the manufacturer. The entire experiment was performed in duplicate.

The meat raw material was acquired in the local commerce in the city of São José do Rio Preto, Brazil contemplating the Federal Inspection Service. Raw meat was purchased for each process (twice), of which there were two processes for each stage of the work (two stages) and the pieces were not frozen so as not to interfere with the functionality of the protein.

All treatments of Italian-type salami were produced with pork shoulder (800 g / kg), pork bacon (200 g/kg), sodium chloride (25 g/kg), sucrose (20 g/kg), garlic powder (3 g/kg), ground black pepper (2 g/kg), nutmeg powder (2 g/kg), cloves powder (2 g/kg), Bactoferm® T-SPX starter culture (0.25 g/kg) (*Staphylococcus xylosus* + *Pediococcus pentosaceus*; Chr-Hansen, Hørsholm, Denmark) and water (0.04 mL/L). The açaí extract and starter culture were previously dissolved in water. All meat raw materials were ground together through an 8 mm diameter grinder. Then, sodium chloride was added to extract the myofibrillar proteins and soon after, the other ingredients, additives and seasonings were incorporated into the meat mass.

After complete homogenization, the meat was embedded in artificial collagen casings with a diameter of 60 mm and tied to obtain pieces of approximately 0.25 kg. After embedding, the pieces were submitted to a maturation chamber with the following temperature and relative humidity programming: 25 °C and 90 % for three days; 23 °C and 88 % for three days; 20 °C and 85 % for three days; 18 °C and 80 % for five days and 15 °C and 75 % until day 21. Air velocity was kept below 0.5 m/sec. Maturation ended when the pieces showed < 0.91 water activity (21 days). The analyses were carried out during ripening time until 21 days with sampling points in 1, 3, 7, 16 and 21 days.

2.3. Water activity (Aw)*, Weight loss and Microbiological analysis (LAB)*

Water activity (Aw) was determined using AquaLab 4TE (Decagon Devices Inc., Pullman, USA) with a reading range from 0.03 to 1.00 ± 0.003 of water activity. For weight loss was used three units of Italian-type salami for each treatment and they were weighed on day 1 of ripening and reweighed on days 3, 7, 16 and 21. The results were calculated through the difference between the initial and the final weight and expressed

in percentage (GLISIC et al., 2019). Lactic acid bacteria (LAB) were counted after inoculation on Man Rogos Sharpe (MRS) agar (Difco™, United Kingdon) (pH 5.6) and incubation at 30 °C for 48 h under anaerobic condition, performed in all treatments (BEDIA; MÉNDEZ; BAÑÓN, 2011).

2.4. Instrumental color Italian-type salami and pH determination

The color parameters were determinate on a ColorFlex45/0 colorimeter (Hunterlab, Reston, USA) calibrated with black and white glasses. Samples were measured using D65 as illuminant, aperture size of 7.5 mm and viewing geometric angle of 10°. The color specification system was CIELAB (L*, a* and b*) and parameters such as luminosity (L*), red intensity (a*) and yellow intensity (b*) were evaluated. Ten readings were performed for each treatment.

For pH determination, the analysis was determined in quadruplicate, using a digital pH meter mPA210 (Tecnopon, São Paulo, Brazil) previously calibrated with two standard solutions (pH 4 and pH 7) at room temperature and equipped with a penetration electrode which was direct inserted into the sample

2.5. Lipid oxidation (TBARS assay)

Lipid oxidation was determined from the reaction between the oxidation products with thiobarbituric acid (TBA), generating compounds that can be measured in a spectrophotometer at 532 nm, according to the method described by Vyncke (1975). At first, the samples were completely homogenized. For analysis, briefly, an aliquot of 5g of sample was mixed with 25 ml of trichloroacetic acid solution (7.5 %) for 2 min in Ultra-Turrax (IKA®-Werke GmbH & Co. KG, Staufen, Germany) and centrifuged (Routine 420R, Hettich Zentrifugen, Germany) at 3500 rpm for 10 min. After filtered, 5 ml of supernatant was added to 5 ml of thiobarbituric acid solution 0.02 M and placed in water bath (96 °C for 40 min). The pigments present in açaí pulp and consequently present in the extract are water soluble, so it can be in the supernatant before the addition of the thiobarbituric acid solution, therefore, the determination of TBARS was performed with modifications to prevent interference from pink color as described by (BELLUCCI et al., 2021a). 1,1,3,3 tetraethoxypropane (TEP) was used as a standard and the results were expressed in mg of malonaldehyde (MDA)/kg of sample.

2.6. Chemical composition

Proximate compositions were performed at the end of ripening time (21day). All analyses were performed in triplicate. It was determined following the official method of the Association of Official Analytical Chemistry (AOAC, 2007) for moisture, protein, and ash content. The fat content was determined following the method proposed by Bligh & Dyer (1959). Results were expressed in g/100 g.

2.7. Texture Profile Analysis (TPA) of Italian-type salami

Texture profile analysis was carried out at the end of the ripening (21 days). Samples were cut into cylinders of 2 x 2 cm (height x diameter). Texture parameters (hardness, springiness, cohesiveness, and chewiness) were obtained in a texture analyzer (TA-XT/Plus/50, Godalming, England) previously calibrated using a standard weight of 5 kg and equipped with a cylindric probe (flat surface area of 19.85 cm^2). Data collection and construction of TPA curves were performed using the Texture Exponent 32 program (Stable Micro Systems Godalming, England). The test was conducted with two cycles of 50% compression with a test speed of 1 mm/s to obtain the force-time curve. Ten replications were performed for each treatment.

2.8. Statistical analysis

Five treatments (CON, ERY, EA250, EA500, EA750) of Italian-style salami were produced in triplicate (n = 3) and analyzed during processing (0, 3, 7, 16 and 21 days) and refrigerated storage (0, 5, 10, 15, 20) after slicing. For the statistical analysis of the results, analysis of variance (ANOVA) was performed using the General Linear Model (GLM). Processing, storage, and treatment time were considered fixed effects and repeat manufacturing a random effect. Statistical analyzes were performed using the STATISTICA software version 7 (StatSoft, Inc., 2004).

3. Results and Discussion

3.1. Weight loss, pH, Water activity (Aw) and LAB count

The antioxidants added (sodium erythorbate and açaí extract) do not affect the weight loss parameter ($p > 0.05$) in all sampling point during the ripening time (3, 7, 16 and 21 days) (Table 4-1).

Table 4-1 Weight loss, pH, and water activity of Italian-type salami with sodium erythorbate and açaí extract during ripening time.

Days	CONT	ERY	EA250	EA500	EA750	SEM	Sig.
Weight loss (%)							
3	6.96^{D}	7.71^{D}	7.40^{D}	7.37^{D}	7.10^{C}	0.417	n.s.
7	20.62^{C}	21.10^{C}	22.63^{C}	21.86^{C}	25.84^{B}	0.718	n.s.
16	36.45^{B}	37.23^{B}	37.44^{B}	37.71^{B}	38.42^{A}	0.395	n.s.
21	41.29^{A}	42.28^{A}	42.17^{A}	42.73^{A}	43.04^{A}	0.225	n.s.
SEM	2.997	3.043	3.051	3.010	3.203		
Sig.	*	*	*	*	*		
Water activity (Aw)							
1	0.974^{A}	0.974^{A}	0.974^{A}	0.972^{A}	0.973^{A}	0.001	n.s.
3	0.969^{A}	0.964^{AB}	0.971^{A}	0.968^{A}	0.970^{AB}	0.002	n.s.
7	0.948^{B}	0.952^{B}	0.951^{B}	0.954^{B}	0.954^{B}	0.001	n.s.
16	0.911^{C}	0.922^{C}	0.918^{C}	0.916^{C}	0.914^{C}	0.003	n.s.
21	0.892^{D}	0.885^{D}	0.889^{D}	0.885^{D}	0.887^{D}	0.002	n.s.
SEM	0.006	0.006	0.006	0.006	0.006		
Sig.	*	*	*	*	*		
pH							
1	5.79^{A}	5.78^{A}	5.78^{A}	5.80^{A}	5.79^{A}	0.011	n.s.
3	4.86^{BC}	4.90^{BC}	4.87^{B}	4.87^{BC}	4.82^{BC}	0.017	n.s.
7	4.80^{Ca}	$4.77C^{ab}$	4.79^{Bab}	4.75^{Cbc}	4.71^{Cc}	0.009	*
16	4.91^{B}	5.01^{B}	4.87^{B}	4.92^{B}	4.89^{B}	0.026	n.s.
21	4.82^{Cab}	4.85^{Ca}	4.74^{Bbc}	4.73^{Cbc}	4.71^{Cc}	0.014	*
SEM	0.043	0.077	0.074	0.075	0.078		
Sig.	*	*	*	*	*		

$^{a–b}$ Mean values in the same row (different treatment in the same day) with different letters indicate significant difference ($P < 0.05$); $^{A-D}$ Mean values in the same column (same treatment in different days) with different letters indicate significant difference ($P < 0.05$); SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; *$P < 0.05$. Treatments: CON: without antioxidant; ERY: with sodium erythorbate at 500 mg kg^{-1}; EA250: with açaí extract at 250 mg.kg^{-1}; EA500: with açaí extract at 500 mg.kg^{-1}; EA750: with açaí extract at 750 mg.kg^{-1}.

Therefore, regarding to the effect of the time above the sample, it was possible observe the same trend between the treatments, that is, the samples had an increase of the weight loss during the time (Table 4-1). Weight loss in dry fermented sausages occurs due the loss of water and essential water-soluble substances during the fermentation phase (Coelho et al., 2019). Similar with the results, Wójciak & Dolatowski (2016) found values of weight loss around 41% when evaluated the application of natural preservatives in combination with acid whey in fermented sausage after 21 days of ripening justify meanly for the reduction on pH values which step up the diffusion of water from the inside to the surface of sausage. Santos et al. (2021) also reported an increase in weight losses during the ripening period on processing of fermented sausages added with dietary fiber (microcrystalline cellulose, resistant starch, and oat fiber).

At ripening time, the pH reduces due the water-soluble carbohydrates such as saccharoses being converted into organic acids by lactic acid bacteria (LAB) under anaerobic conditions (LIU et al., 2015). It is possible observe this trend (Table 4-1), after 3 days the pH values decrease from about 5.78 to 4.87 in all treatments, referring to the fermentation phase. However, pH values remained stable in the dryer phase (from 4 to 21 days) and to the end of ripening, the pH values ranged from 4.71 to 4.85. In this regard, Coelho et al. (2019) observe the same behavior where pH values at first time, reduced in fermentation and after kept constant until 21 days followed by an increase in the last seven days caused by accumulation of nitrogen catabolism products and non-protein amino acids. It is interesting to emphasize that pH reduction is an important technological step to fermented meat products and its reduction is capable to inhibit the gown of pathogenic microorganisms (SANTOS et al., 2021). Açai extract affected pH parameters at 7 and 21 days ($p<0.05$), so, the addition of açaí extract at concentrations of 500 and 750 mg kg-1 contributed to the reduction of pH at the end of processing.

Acidification and weight losses is correlates to losses of water by evaporation or dripping which is reflect on decrease of water activity and moisture (COELHO et al., 2019). In this study, water activity parameter does not affect by the addition of açai extract or sodium erythorbate ($p > 0.05$) in all sampling points (Table 4-1). In regarding to time, it was observed a reduction of water activity during ripening ($p<0.05$). At the beginning of ripening (1 day), water activity ranged from 0.972 to 0,974 between the treatments while in the end of the ripening time, ranged from 0.885 to 0.892 and reached lower values than the maximum value allowed by Brazilian legislation for this product (< 0.9)

(BRASIL, 2000). Similar water activity results were obtained by Ruiz et al. (2014) in Italian fermented sausage. It is known that a reduction on pH values reaching or approaching the isoelectric point of proteins there is a decrease of water retention capacity.

Regarding to LAB count (Table 4-2), in the beginning of fermentation step and ripening all treatments presented similar values (5 to 5.5 Log CFU/g) ($p > 0.05$). However, with 3 days of fermentation, the LAB count was affected by the addition of açaí extract ($p<0.05$), which EA500 and EA750 treatments presented higher value (9 Log CFU/g) than the other (7.85, 7.70 and 8 Log CFU/g to CON, ERY and EA250, respectively). At the end, no difference was observed between the treatments and the values of LAB count ranged from 8.07 to 8.65 Log CFU/g, but the higher values are also presented by EA500 and EA750. Açaí extract in concentrations higher than 500 $mg.kg^{-1}$ shows to influence the count of lactic acid bacteria due the presence of sugar in its composition, which can promote functionality beneficial to the consumer's health. In a study conducted by Santos et al. (2021), which investigated the addition of fiber in dry fermented meat product, sugars and glycosidic bonds promoted the highest values in the LAB population count.

Table 4-2 Lactic acid bacterial (LAB) count of Italian-type salami with sodium erythorbate and açaí extract during ripening time.

Microbiological analysis (LAB) (Log CFU/g)								
	Days	**CONT**	**ERY**	**EA250**	**EA500**	**EA750**	**SEM**	**Sig.**
	1	5.00^{B}	5.00^{B}	5.00^{C}	5.50^{B}	5.00^{B}	0.100	n.s.
	3	7.85^{bA}	7.70^{bA}	8.00^{bB}	9.00^{aA}	9.00^{aA}	0.192	*
	21	8.07^{A}	8.15^{A}	8.39^{A}	8.65^{A}	8.65^{A}	0.143	n.s.
SEM		0.630	0.623	0.678	0.735	0.814		
Sig		*	*	*	*	*		

$^{a–b}$ Mean values in the same row (different treatment in the same day) with different letters indicate significant difference ($P < 0.05$); $^{A-D}$ Mean values in the same column (same treatment in different days) with different letters indicate significant difference ($P < 0.05$); SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; *$P < 0.05$. Treatments: CON: without antioxidant; ERY: sodium erythorbate at 500 mg kg^{-1}; EA250: açaí extract at 250 $mg.kg^{-1}$; EA500: açaí extract at 500 $mg.kg^{-1}$; EA750: açaí extract at 750 $mg.kg^{-1}$.

3.2. Color Parameters

Regarding to color parameters, the values of L*, a* and b* are shown in Table 4-3. Differences in L* value was found between the treatments in each sampling time ($P < 0.05$), except on day 3. So, lightness was affected by treatments. Particularly on day 0, treatments CONT and ERY showed the highest values of lightness while treatments with açai extract (EA250, EA500 and EA750) showed the lowest values, not differing from each other. On day 7, the EA500 treatment presented the highest value fallowed by the other treatments, that did not differ from each other. Finally, at 16 days, the ERY treatment had the highest value and at 21 days, the highest L* values were presented by CONT and EA250 treatments, showing to be the clearest treatments at the end of processing. In a study carried out by Bellucci et al. (2022), açaí extract reduce L* color parameter when added in pork patties. Over the time, there was an increase of L* values from day 0 to 7 day, followed by a decrease trend until the end of ripening. According to Ruiz et al., (2014), the decline of lightness is consequence of curing, drying and maturation process due the concentration of solids caused by dehydration. That agrees with to Lorenzo et al. (2013), who observed a decrease of L* values in the processing phase of dry cured sausage, affirm that the reduction of lightness during the ripening time can be attributed to the water losses.

Redness was affected by both time of ripening and treatments ($P < 0.05$). At the first day, ERY presented the highest a* value and EA500 and EA750 treatments the lowest values, not differing from each other. In general, a* value was affected by the addition of antioxidants, where the treatment with sodium erythorbate in its formulation (ERY) showed the highest values of a* color paraments while the addition of 500 and 750 $mg.kg^{-1}$ of açaí extract negatively affect his parameter, shown the lowest values in all sampling points. However, over the time., EA500 and EA750 presented a significant increase during the ripening time, ranged from 8.98 and 8.30 at first day to 10.51 and 10.80 at 21 days, respectively. Color is the most important attribute in meat products such as dry fermented sausage or Italian-style sausage, as it is one of the main parameters that influence consumers' purchase intention and the color formed during ripening (brick-red) makes it more attractive, therefore, natural antioxidants could provide more attractive sausages (LORENZO et al., 2013).

Results shown that b* values also ate affected both ripening time and treatments ($p<0.05$). The açaí extract addiction also affected this parameter in all sampling points

AE500 and AE750 presented the lowers values of b*, while in most of time ERY showed the higher followed CON and EA250. In regarding to açaí extract, when added as natural antioxidant in pork patties, was observed that an increase in the concentration of AE resulted in lower b* values (BELLUCCI et al., 2022). Over the ripening time, yellowness decreases in all treatments ($p<0.05$). This outcome agrees with Ruiz et al. (2014) who observed the same trend in Italian salami sausage. The authors state that this drop in b* values is attributed to the consumption of oxygen by microorganisms during their exponential growth phase promoting a reduction in oxymyoglobin.

Table 4-3 Color parameters of Italian-type salami with sodium erythorbate and distinct levels of açaí extract (EA) during processing.

Days	CON	ERY	EA250	EA500	EA750	SEM	Sig.
L*							
1	47.32^{abC}	48.63^{aC}	45.69^{bcC}	44.91^{cD}	46.55^{bcC}	0.282	*
3	57.63^{A}	57.61^{A}	57.24^{A}	58.31^{B}	57.70^{A}	0.141	n.s.
7	$57.76b^{A}$	57.84^{bA}	57.72^{bA}	59.76^{aA}	57.45^{bA}	0.133	*
16	50.89^{bB}	52.96^{aB}	49.83^{bB}	49.75^{bC}	50.70^{bB}	0.182	*
21	48.94^{aC}	46.30^{bD}	48.78^{aB}	46.39^{bD}	45.44^{bC}	0.219	*
SEM	0.281	0.316	0.358	0.413	0.379		
Sig.	*	*	*	*	*		
a*							
1	10.35^{bA}	11.66^{aA}	10.42^{bA}	8.98^{cBC}	8.30^{cC}	0.132	*
3	9.65^{bB}	10.26^{aB}	10.30^{aA}	9.40^{bB}	9.74^{bB}	0.061	*
7	9.97^{abB}	10.43^{aB}	9.52^{bcB}	8.67^{dC}	9.35^{cB}	0.069	*
16	10.85^{aA}	10.85^{aB}	10.81^{aA}	10.25^{bA}	9.42^{cB}	0.067	*
21	10.46^{bA}	11.68^{aA}	10.30^{bA}	10.51^{bA}	10.80^{bA}	0.094	*
SEM	0.061	0.097	0.080	0.074	0.087		
Sig.	*	*	*	*	*		
b*							
1	15.03^{aA}	15.06^{aA}	13.97^{bA}	12.54^{bA}	10.35^{cB}	0.136	*
3	11.28^{bB}	11.97^{aB}	11.68^{abB}	10.86^{cBC}	10.60^{cAB}	0.056	*
7	11.64^{abB}	$11.59^{ab}C$	11.68^{aB}	11.27^{bB}	10.88^{cA}	0.047	*
16	10.56^{bC}	11.22^{aD}	10.25^{bC}	10.38^{bC}	9.31^{cC}	0.055	*
21	$9.80b^{D}$	10.25^{aE}	9.91^{abC}	$9.72b^{cD}$	9.31^{cC}		
SEM	0.113	0.101	0.082	0.080	0.064		
Sig.	*	*	*	*	*		

$^{a–b}$ Mean values in the same row (different treatment in the same day) with different letters indicate significant difference ($P < 0.05$); $^{A-D}$ Mean values in the same column (same treatment in different days) with different letters indicate significant difference ($P < 0.05$); SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; *$P < 0.05$. Treatments: CON: without antioxidant; ERY: sodium

erythorbate at 500 mg kg^{-1}; EA250: açaí extract at 250 $mg.kg^{-1}$; EA500: açaí extract at 500 $mg.kg^{-1}$; EA750: açaí extract at 750 $mg.kg^{-1}$.

3.3. Lipid oxidation during ripening

Lipid oxidation was determinates through the quantification of substances that reacts with thiobarbituric acid (TBARS) (Table 4-4), and no significant difference was found in lipid oxidation during the ripening time, that is, time did not affect TBARS values, except to ERY treatment. However, at 3 and 21 days, the lipid oxidation presented significant difference between treatments ($p < 0.05$), where CON shown the higher values of TBARS (0.26 and 0.33 mg malonaldehyde/kg of sample at 3 and 21 days, respectively) while treatments with AE added (EA250, EA500 and EA750) no differ from ERY (sodium erythorbate) and this result evidence the antioxidant potential of açaí extract against lipid oxidation. Bellucci et al. (2022) also reported an antioxidant potential from açai extract when applied in pork patty. According to Matta et al. (2020), açaí fruit only has a lower anthocyanin content than blackcurrant, blueberry and blackberry and cyanidin-3-glucoside and cyanidin-3-rutinoside were the major anthocyanins in purple açaí. Beside these anthocyanins, rutin, orientin, homoorientin, catechin, epicatechin, ferulic acid, vanillicacid, gallic acid, p-hydroxybenzoic acid and syringic acid are also found is açai pulp. These bioactive are linked with its high antioxidant activity because these compounds have higher potential of free radical-scavenging and provide to the pulp a higher oxygen radical absorbance capacity (Carvalho et al., 2017; Garzón et al., 2017).

In this study, TBARS values ranged from 0.18 to 0.20 mg MDA/kg between sodium erythorbate (ERY) and AE (EA250, EA500 and EA750) treatments at the end of ripening. Faria et al. (2020) found similar values to lipid oxidation at the end of ripening time in Italian salami added of sea asparagus extract and reduction of curing salt (0,22 and 0.19 mg MDA/kg) although the control presented lower value than in this study (0.18 vs. 0.33 mg MDA/kg). Abu Salem & Ibrahim (2010) reported higher TBARS values in dry fermented buffalo sausage 15 days in processing (ripening), but also observe that sage oil extract could reduce lipid oxidation. The maximum TBARS value found is lower than that reported by Šojić et al. (2020) as the limit value acceptable, that is, above 0.5 mg malonaldehyde/kg the oxidation products are already capable of compromising the quality and acceptance of the product.

Table 4-4 Lipid oxidation values of Italian-type salami with sodium erythorbate and distinct levels of açaí extract (EA) during processing (mg MDA/kg of sample).

Days	CON	ERY	EA250	EA500	EA750	SEM	Sig.
1	0.18	0.13^{B}	0.16	0.18	0.18	0.008	n.s.
3	0.26^{a}	0.21^{abAB}	0.19^{b}	0.17^{b}	0.21^{ab}	0.009	*
7	0.25	0.25^{A}	0.23	0.26	0.25	0.024	n.s.
16	0.29	0.24^{AB}	0.21	0.24	0.23	0.013	n.s.
21	0.33^{a}	0.18^{bAB}	0.19^{b}	0.18^{b}	0.20^{b}	0.019	*
SEM	0.025	0.018	0.010	0.016	0.016		
Sig.	n.s.	*	n.s.	n.s.	n.s.		

[a–b] Mean values in the same row (different treatment in the same day) with different letters indicate significant difference ($P < 0.05$); [A-B] Mean values in the same column (same treatment in different days) with different letters indicate significant difference ($P < 0.05$); SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; * $P < 0.05$. Treatments: CON: without antioxidant; ERY: sodium erythorbate at 500 mg kg^{-1}; EA250: açaí extract at 250 mg.kg^{-1}; EA500: açaí extract at 500 mg.kg^{-1}; EA750: açaí extract at 750 mg.kg^{-1}.

3.4. Chemical Composition and Texture Analyses Profile at the end of ripening

The effect of açaí extract concentration on the proximate composition of Italian-type salami is shown in Table 4-5. No differences were observed for moisture, protein, fat, and ash ($P>0.05$) between the treatments. These results showed that both açaí extract and sodium erythorbate did not interfere with the proximate composition of dry fermented sausage and its results corroborate the data reported by Bellucci et al. (2022), who found no significant differences in the chemical composition of pork hamburgers made with the addition of açaí extract and sodium erythorbate, and de Carvalho et al. (2020) who observed that turmeric extract did not affect the proximate composition of fresh lamb sausage with the addition of turmeric extract. Regarding to moisture, the values ranged from 32.37 to 33.97 g/100g and these results are similar to those found by Smit et al. (2020) at the end of ripening of Italian salami with honeybush extract in its formulation (ranged from 33.5 to 35.3%). The authors also reported that these results are within the range moisture for typical commercial Italian salami (24.3%–53%).

The instrumental texture parameters are shown in Table 4-5. Hardness was not significantly affected ($P > 0.05$) by the addition of antioxidants, however, cohesiveness springiness and chewiness parameters were affected by the addition of AE ($p<0.05$). Springiness showed the lowest values in treatments EA250 and EA750. Cohesiveness

and chewiness had similar behavior, the lower values were presented by treatments with AE, which the was EA250, followed by EA750 and EA500, in this order and the higher were shown in CON and ERY treatments. Faria et al. (2020) found that texture of Italian salami was not affected by sea asparagus extract when compared to control.

Table 4-5 Centesimal composition (g/100g) and instrumental texture profile of Italian-type salami with sodium erythorbate and different levels of açaí extract (EA).

Parameter	CON	ERY	EA250	EA500	EA750	SEM	Sig.
Chemical composition							
Moisture	33.97	32.94	33.12	32.76	32.37	0.389	n.s.
Protein	28.59	25.25	29.15	28.82	26.18	0.186	n.s.
Lipid	20.27	26.15	22.12	25.66	20.69	1.266	n.s.
Ash	4.94	5.21	4.65	5.53	4.96	2.653	n.s.
TPA							
Hardness (N)	42.80	42.62	42.66	48.02	48.55	0.905	n.s.
Cohesiveness	0.42^{a}	0.39^{ab}	0.36^{c}	0.38^{bc}	0.37^{bc}	0.004	*
Chewiness (N.mm)	9.51^{ab}	9.61^{a}	7.56^{c}	9.37^{b}	8.71^{bc}	0.217	*
Springiness (mm)	0.53^{a}	0.50^{abc}	0.49^{bc}	0.52^{ab}	0.48^{c}	0.004	*

$^{a–b}$ Mean values in the same row (different treatment in the same day) with different letters indicate significant difference ($P < 0.05$). SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; *$P < 0.05$. Treatments: CON: without antioxidant; ERY: sodium erythorbate at 500 mg kg^{-1}; EA250: açaí extract at 250 $mg.kg^{-1}$; EA500: açaí extract at 500 $mg.kg^{-1}$; EA750: açaí extract at 750 $mg.kg^{-1}$.

4. Conclusion

The addition of açaí extract powder (500 and 750 $mg.kg^{-1}$) improved the oxidative stability, reduced the pH, no affected the final water activity, and increased the growth of lactic acid bacteria in Italian-style salami in the fermentation time. Therefore, they can be used as a natural antioxidant to improve the quality and extend the shelf life of Italian-type salami.

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CONCLUSÃO GERAL

O extrato obtido da polpa de pitaia vermelha, quando adicionado em hambúrguer suíno com substituição total da gordura suína por emulsão de óleo de chufa mantido sob refrigeração e incidência de luz, foi capaz de reduzir as reações de oxidação, melhorar a estabilidade da cor e aumentar a aceitação sensorial do produto. Mostrando-se, assim, ser uma alternativa aos antioxidantes sintéticos.

Em relação ao efeito do extrato de açaí na estabilidade de cor de hambúrguer suíno refrigerado conclui-se que seu uso preveniu a descoloração durante o armazenamento. O extrato de açaí tornou as amostras mais escuras, no entanto preservou a intensidade de cor vermelha ao passo que o aumento das concentrações adicionadas reduziu a intensidade de cor amarela dos hambúrgueres. A estabilidade oxidativa também foi melhorada com a adição do extrato de açaí, visto que durante o armazenamento, os resultados de oxidação lipídica dos tratamentos com extrato de açaí foram iguais aos apresentados pelo adicionado de eritorbato de sódio. A aplicação de 250 mg/kg de extrato reduz a oxidação lipídica tanto quanto a de eritorbato de sódio a 500 mg/kg, comprovando o potencial antioxidante do extrato estudado em hambúrguer suíno refrigerado, sendo possível ser aplicado em outros produtos cárneos.

Em salame tipo italiano avaliado durante o período de processamento, o extrato de açaí não influenciou perda de peso e atividade de água, mas sua aplicação nas concentrações de 500 e 750 mg/kg aumentou a contagem de bactérias ácido lácticas durante a fermentação e reduziu dos valores de pH ao final do período de processamento, parâmetros importantes para produtos cárneos fermentados. O extrato de açaí melhorou a estabilidade oxidativa em salame tipo Italiano tanto quanto o eritorbato de sódio, pois os menores valores de oxidação lipídica foram apresentados pelos tratamentos adicionados de antioxidantes ao final do processamento. Estudos devem ser conduzidos para avaliar o efeito do extrato de açaí em salame tipo Italiano também durante a vida útil. Portanto, nas concentrações estudadas, tanto o extrato de pitaia quanto o de açaí podem ser utilizados como antioxidantes para melhorar a vida útil de produtos cárneos.

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de São José do Rio Preto

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