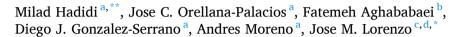
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# Plant by-product antioxidants: Control of protein-lipid oxidation in meat and meat products



<sup>a</sup> Department of Organic Chemistry, Faculty of Chemical Sciences and Technologies, University of Castilla-La Mancha, 13071, Ciudad Real, Spain

<sup>b</sup> Centre D'Innovació, Recerca I Transferència en Tecnologia Dels Aliments (CIRTTA), TECNIO-UAB, XIA, Department de Ciència Animal I Dels Aliments, UAB-Campus,

Universitat Autònoma de Barcelona, 08193, Bellaterra, Spain

<sup>c</sup> Centro Tecnológico De La Carne de Galicia, Avd. Galicia Nº 4, Parque Tecnológico de Galicia,San Cibrao Das Viñas, 32900, Ourense, Spain

<sup>d</sup> Universidade de Vigo, Área de Tecnología de Los Alimentos, Facultad de Ciencias de Ourense, 32004, Ourense, Spain

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### ABSTRACT

Protein-lipid oxidation is one of the main causes of quality deterioration in meat and meat products during processing and storage. The application of natural antioxidants in muscle food appears a sustainable option for reducing the consumption of synthetic antioxidants with confirmed carcinogenic and toxicological effects. Hence, the food industry today prefers low-cost natural additives instead of synthetic ones. Agro-food industry generates a large quantity of plant by-products annually during the cultivation and processing of agricultural products. There is a wide variety of natural antioxidants in plant by-products. Several parts of plant (seeds, peels, leaves, husks, stems, and roots) as unexploited novel sources of natural antioxidant can be applied either through technological strategies to control oxidative process in meat and meat products. This paper provides an overview of the current trends in the use of natural antioxidants from plant by-products for potential applications against protein-lipid oxidation in muscle food. In addition, the effect of encapsulation of plant by-product antioxidants on the protein-lipid oxidation of meat and meat products is reviewed.

# 1. Introduction

During the last 70 years, due to their health benefits and increasing consumer awareness, the international markets have seen a rapid growth in demand for dietary supplements fortified with bioactive compounds such as meat and meat products. Meat is staple diet rich in vital nutrients, including high-quality proteins, vitamins, bioactive compounds, carbs, minerals and colors, and contains varying proportions of storage (triacylglycerols) and structural lipids (phospholipids) depending on the muscle type (Amoli et al., 2021; Cheng et al., 2020). Besides, the global meat consumption market research results show that the market size has increased annually by 58% over the 20 years up until 2018 to reach 360 million tones with 54% of this increase being attributed to population growth, and the rest to increased consumption per capita due to changes in consumers' dietary attributes and incomes (Whitnall & Pitts, 2019). The consumption of meat products in industrialized countries fulfills most of a normal healthy individual's daily protein requirement.

One of the major issues faced by meat factories is providing goods with pleasant flavor and color, as well as freshness attributes that can be maintained throughout the shelf life of meat and meat products at the lowest possible cost (Cmlp & Cyc, 2021). Nevertheless, lipid oxidation during processing and storage leads to a decline in meat products' quality attributes (Domínguez et al., 2019). Oxidation is the main factor responsible for acceptability and quality in meat products or deterioration of their flavor and taste. A high concentration of unsaturated lipids, heme pigments, metal catalysts and oxidizing agents in muscle tissues makes the meat prone to oxidative degradation (Domínguez et al., 2019). The symptoms of oxidative deterioration in meat and meat products include discoloration, off-flavor development, formation of toxic compounds, poor shelf-life, and nutrient and drip losses (Amoli et al., 2021). Lipid-protein oxidation, in addition to nutritional

Corresponding author.

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<sup>\*</sup> Corresponding author. Centro Tecnológico De La Carne de Galicia, Avd. Galicia Nº 4, Parque Tecnológico de Galicia, San Cibrao Das Viñas, 32900, Ourense, Spain.

E-mail addresses: milad.hadidi@uclm.es (M. Hadidi), jmlorenzo@ceteca.net (J.M. Lorenzo).

deterioration, leads to the generation of cytotoxic and genotoxic compounds, which are harmful to human health (Domínguez et al., 2021). One of the fundamental elements required for oxidative metabolic reactions in creation of energy in the living organisms is oxygen. Molecules of oxygen are subjected to a series of reactions that may result in the generation of free radicals under normal physiological conditions. As part of the metabolic reaction, two to five percent of the oxygen consumed is converted into reactive oxygen species. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) regulate several homeostatic processes by interacting with proteins, fatty acids, and nucleic acids. Numerous chronic disorders like cancer, deficiency in immunity, cardiovascular diseases (CVDs) and ageing are associated with oxidative stress (Falowo et al., 2014).

Antioxidants, as hydrogen atom donors, are used in meat products, aiming to retard or prevent from undergoing oxidative reactions. The majority of the antioxidants added to animal products such as meat are synthetic; however, due to the current trend to minimize the use of synthetic food additives and since natural components have become increasingly popular, further research is needed to uncover novel and natural extracts with potential applications for meat and meat products (Lorenzo et al., 2018; 2019). In order to combat the challenges of the oxidative instability of lipids and proteins in meat, it is necessary to explore natural alternatives, including plant-derived antioxidants for their low cost and health benefits (Munekata et al., 2020). Agro-food industry generates a large quantity of plant by-products annually during the cultivation and processing of agricultural products. There is a wide variety of natural antioxidants in plant by-products. Several parts of plant including seeds, peels, leaves, husks, stems, and roots as unexploited novel sources of natural antioxidant. The valorization of by-products with the recovery of antioxidant rich extracts is even more interesting knowing the fact that the non-edible parts of fruits and vegetables often contain a higher bioactive contents than the edible parts (Echegaray et al., 2018; Lourenço et al., 2019). Tocopherols, carotenoids and phenolic compounds are bioactives in plant sources that are classified as "Generally recognized as safe" (GRAS) food additives for human consumption with pharmaceutical, antimicrobial, and antioxidant properties by the US Food and Drug Administration (FDA) (Brandolini et al., 2022). As a consequence, a viable alternative from natural sources like plant-derived antioxidants is needed to tackle the difficulties of lipid and protein oxidative instability in meat and meat products (Pateiro et al., 2021). In this regard, encapsulation technology is defined as a procedure to entrap bioactives within small capsules as a material wall which is a useful tool to improve the delivery of bioactives into meat products and can release their contents at controlled rates over prolonged periods under specific conditions (Hesami et al., 2022; Majidiyan et al., 2022). Hence, the objective of the present review was studying the application of bioactive natural compounds in plant by-products as natural antioxidants to delay oxidative reaction in these products.

#### 2. Plant by-products as a source of natural antioxidants

Globally, approximately 1.3 billion tons of food, including meat is spoiled or wasted per year throughout the supply chain, from production down to final household consumption. However, this massive wastage, which has become a major concern to consumers, governments and food industries, is associated with the outbreak of foodborne diseases. In the past decades, a small percentage of the wastages of meat and meat products was used for livestock and poultry feed, but in recent years, they have found multiple functions in the food and cosmetic industries (Salami et al., 2016). Close to 50% of the total meat spoilage and wastage occurs at the household consumption level due to poor preservative techniques and facilities. Meat wastages are caused through microbial and chemical spoilage with the consequence of food-borne illnesses, economic loss and food insecurity (Domínguez et al., 2019). products due to the restrictive regulations on the consumption of these additives and consumer awareness about their toxic effect and health risk. Plant by-product additives offer natural antioxidants with phenolic compounds that are a complex group formed by the metabolism of plants. Among the best sources of plant-derived antioxidants are fruits, vegetables, spices, herbs, cereals, grains, and seeds. As shown in Fig. 1, several parts of plants such as seeds, peels, leaves, husks, stems, and roots (as unexploited novel sources of natural antioxidants) can be used either through technological strategies to control the oxidative process in meat and meat products, and thus, avoid the financial and environmental problems of these by-products (Domínguez et al., 2020; Nikmaram et al., 2018).

Antioxidant-containing foods would stay fresh longer and their desired sensory properties will be preserved by preventing oxidation, decomposition and discoloration during storage (Tomasevic et al., 2021). Bioactive compounds can be found in abundance in plants like seeds, grains, spices, herbs, cereals, vegetables, and fruits. There are many of components that can restrain oxidation, but only a small percentage of them are safe to consume due to toxicological concerns. Regulatory agencies must approve food grade antioxidants to meet the safety criteria (GRAS). Moreover, from an applicative viewpoint, they must not adversely influence color, odor or taste, should be effective at low concentrations (1-100 mg/100 g) of agents, be compatible with food, be easily applicable, be stable during processing and shelf-life, and be economically viable. Above important, such substances and their metabolites must be harmless at higher concentrations than those seen in typical meals. Addition of natural antioxidant extracts has also been shown to enhance meat softness (Pateiro et al., 2018).

#### 3. Protein and lipid oxidation mechanisms in meat

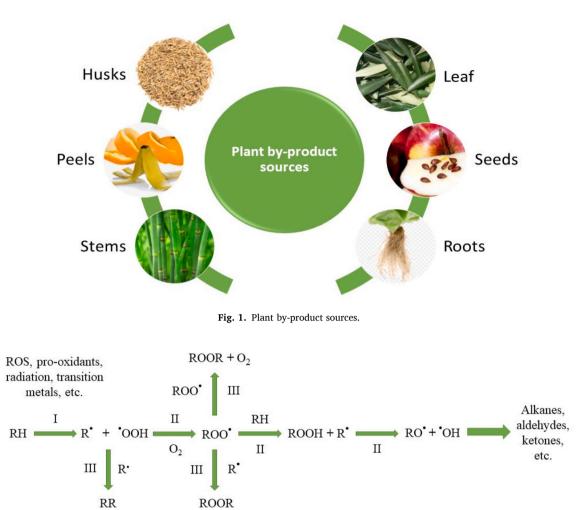
Living tissues have a multitude of reactions that help in the regulation of cellular homeostasis. Some of them correspond to oxidationreduction reactions that control the number of certain compounds. These free radicals are known as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can accumulate in the absence of post-mortem homeostatic mechanisms in tissues and interact with the molecules like proteins, fatty acids and alike. These interactions produce oxidation by-products that can be a drawback in the sectors such as the meat industry; this, in turn, can decrease the shelf-life of meat products, resulting in sensory changes and formation of toxic substances that are potentially harmful to consumers. So, the second most important cause of meat spoilage is lipid and protein oxidation (Manessis et al., 2020).

# 3.1. Lipid oxidation

The phenomenon known as lipid oxidation can be catalyzed for several reasons such as the presence of ROS, RNS and metallic ions. During this process, unsaturated fatty acids and oxygen interact with each other indirectly because oxygen previously needs to be activated. Usually, this activation results in the formation of hydroxyl radicals ( $^{\circ}$ OH), superoxide anions radicals ( $O_2^{\bullet-}$ ) or hydrogen peroxides (H<sub>2</sub>O<sub>2</sub>). The singlet oxygen facilitates the formation of ROS (Domínguez et al., 2019).

There are several factors influencing the above oxygen activation, including energy sources (light and temperature), pro-oxidant molecules, existence of previous ROS, transition metals, etc. Depending on how the activation takes place, a distinction can be made between autoxidation and photo-oxidation, but there is also enzymatic-catalyzed lipid oxidation; the most important of them for meat industry is autoxidation as it is the main path in which unsaturated fatty acids and oxygen come into contact, leading to the deterioration of meat and meat products (Falowo et al., 2014).

Lipid oxidation (Fig. 2) takes place in three stages (Manessis et al., 2020):



**Fig. 2.** General lipid oxidation mechanism: I) initiation, II) propagation, and III) completion RH: unsaturated fatty acid, ROS: reactive oxygen species, R<sup>•</sup>: alkyl radical, RR: non-radical product, ROO<sup>•</sup>: peroxyl radical, ROOR: organic peroxide, ROOH: hydroperoxides, <sup>•</sup>OOH: hydroperoxyl radical, RO<sup>•</sup>: alkoxy radical, <sup>•</sup>OH: hydroxyl radical, and O<sub>2</sub>: oxygen.

- I. Initiation: an unsaturated fatty acid is stripped of a hydrogen atom, resulting in the formation of an alkyl radical, which can either react with another alkyl radical to form a non-radical product or continue to the propagation stage.
- II. Propagation: Alkyl radicals react with molecular oxygen to form peroxide radicals, which are highly reactive and can interact with unsaturated fatty acids (removing a hydrogen atom from them) to form hydroperoxides and more alkyl radicals (primary products). In addition, peroxide radicals can react with other peroxide radicals or alkyl radicals to form organic peroxide. The hydroperoxides formed also decompose, resulting in the formation of alkoxy radicals and hydroxyl radicals. Sometimes, alkoxy radicals decompose to form volatile aromatic compounds like aldehydes, ketones, and alkanes (secondary products). All of these freeradical chain reactions can result in increase of lipid oxidation.
- III. Completion: Reactions between free radicals may give rise to non-radical compounds, and thus, halt their own propagation. Reactions between free radicals and antioxidants also take place in this stage.

## 3.2. Protein oxidation

Due to the presence of large number of proteins in the cells, extracellular tissues and body fluids, as well as their ease of interaction with oxidizing compounds, the phenomenon known as protein oxidation is very common. The importance of protein oxidation towards cellular homeostasis derives from the fact that proteins serve vital roles in regulating cell structure, cell signalling, and the various enzymatic processes of the cell. Protein oxidation can therefore rapidly contribute to oxidative stress by directly affecting cell signalling, cell structure, and enzymatic processes such as metabolism. There are many different modes of inducing protein oxidation including metal catalyzed oxidation, oxidation induced cleavage, amino acid oxidation, and the conjugation of lipid peroxidation products. There is evidence that the metalcatalyzed oxidation of proteins is one the most common mechanisms for inducing protein oxidation, practically for the introduction of carbonyl groups (Cecarini et al., 2007). Just until recently, this process had not been given the same importance as lipid oxidation maybe due to the fact that, although proteins can easily react with oxidizing compounds, the kinetic of their reactions is slower compared to that of lipids, which is why there is scarcity of studies on this subject today (Lorenzo et al., 2018). The protein oxidation process also depends in part on the lipid oxidation process, since the latter produces a multitude of radicals that can react with proteins (Nawaz et al., 2022). When proteins are modified, what usually occur are changes in the amino acid composition of the protein chain, protein polymerization and/or loss of proteolytic activity. Among the amino acids, the most prone to oxidation are arginine, cysteine, histidine, lysine, methionine, phenylalanine, proline, tryptophan, and tyrosine (Domínguez et al., 2021).

These changes can be reversible or irreversible. In general,

permanent modifications in the amino acids of a protein can lead to serious diseases. A wide range of aging-related diseases is at least in part associated with protein oxidative damage and modifications of amino acids. These include eye diseases, metabolic disorders like diabetes and obesity, inflammatory conditions such as arthritis, cardiovascular complications like atherosclerosis, kidney disorders, respiratory disease, cancer, and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (Santos & Lindner, 2017). Free radicals can remove a hydrogen atom from a protein and form a protein radical, which subsequently, reacts with oxygen to form an analkyl proxyl radical (Fig. 3); this, in turn, can either react with a water molecule to give alkoxyl radicals, oxygen, and more water, or react with another protein to give alkoxyl radicals (Manessis et al., 2020). The latter can react with reduced forms of transition metals to form more alkoxyl radicals, which will, ultimately, be transformed into hydroxyl derivatives that cause protein carbonylation (Guyon et al., 2016). Some treatments in meat industry like physical or heat treatments release transition metals like Fe, Cu or Mn from the metalloenzymes present in meat, which are, as mentioned above, important initiators of protein oxidation (Domínguez et al., 2021).

## 4. Control of protein-lipid oxidation in meat and meat products

As mentioned in the previous section, lipid oxidation, and to a lesser extent, protein oxidation, cause the deterioration of meat and meat products, which can, ultimately, alter their properties like shortening their shelf-life and making them harmful to health. However, there are ways to control these phenomena by neutralizing free radicals and ROS present in the cells, and so preventing them from producing chain reactions. The most effective way to do this is using antioxidants (Das et al., 2020; Velázquez et al., 2021).

Just as there are molecules that accelerate the phenomenon of lipid oxidation, there are other molecules (i.e., antioxidants) that are able to slow it down, too. Although not all of them have the same mechanisms of action, the result is the same: to interfere with the formation of highly reactive radicals forming more stable products that limit oxidation process for as long as possible (Domínguez et al., 2019). To do this, antioxidants can donate a hydrogen atom to the free radicals, react directly with oxygen, or scavenge the metal ions capable of catalyzing oxidations. These compounds are naturally present in the living tissues and can slow down the oxidations; when death occurs, by decreasing the number of antioxidants, the number of reactive compounds increases. Similarly, in the meat industry, when an animal is slaughtered, the oxidation process will end up, triumphing over the antioxidants naturally present in it. Some strategies used to achieve this are the use of antioxidants directly to preserve meat or meat products, or feeding the would-be-slaughtered animals with a diet rich in antioxidants (Echegaray et al., 2020Antioxidants, which are present in many foods, can be classified in several ways, but the one that will be used here is being synthetic or natural. This classification is based on whether the antioxidant compound is obtained from a natural source or from a synthetic source. Synthetic antioxidants have long been used as a tool to prevent oxidation in different foods, including meat and meat products. The most common compounds used for this were propyl gallate (PG), octyl gallate (OG), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and butylated hydroxytoluene (BHT). Although they are effective, nowadays there is a process of replacing them with natural antioxidants due to problem of their accumulation in the human body that can lead to allergies or diseases (Gulcin, 2020). Natural antioxidants can be classified based on their origin (animal-based, plant-based, or even bacteria-based) and their structure. Plant-based antioxidants are very interesting because there are many different sources of them with different characteristics (Manessis et al., 2020). This review delves more deeply into the latter type since natural antioxidants are much safer and healthier to the society.

# 4.1. Mechanism of action of plant antioxidants

The polyphenols as natural antioxidants have high radicalabsorbance capacity or have strong hydrogen atom-donating activity. The major antioxidative polyphenols are phenolic acids, flavonoids, and essential oils. Some polyphenols control the creation of free radicals and propagation of ROS, whereas other scavenge free radicals and chelate transition metals. Phenolic acids and flavonoids scavenge free radicals and chelate metals ions such as  $Fe^{2+}$ ,  $Fe^{3+}$ ,  $Zn^{2+}$  and  $Cu^{2+}$  (Fig. 4). The antioxidant activity of the phenolic compounds attributed to their skeleton structure and pattern of functional groups (Kumar et al., 2015). For example, the location and number of free hydroxyl (-OH) groups on flavonoid skeleton decide the free radical scavenging activity. Presence of multiple -OH groups and ortho-3,4-dihydroxy structures improve the antioxidant activity of polyphenols. Polymeric structures (containing more -OH groups) have more antioxidant potential, whereas glycosylation of functional groups like reduction of -OH groups usually decrease antioxidant activity. Anthocyanins and their hydrolyzed products contain -OH groups, which can donate hydrogen atom and thus possess antioxidant potential. Some phenolics include vicinal -OH groups bounded to aromatic ring. These compounds donate hydrogen atom and vicinal -OH groups that can chelate metals, hence inhibit oxidation by more than one way (Choe & Min, 2009).

#### 4.2. Prevention of lipid oxidation

In relation to these compounds, it is very necessary to know their capacity to stop lipid oxidation partially or totally in order to be used. For this purpose, the most common method in meat industry is TBARS (thiobarbituric acid reactive substances) assay. It is based on the

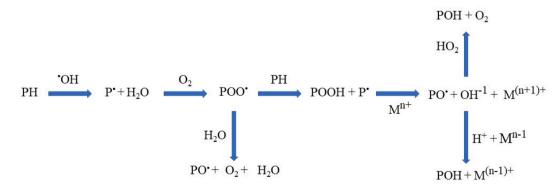
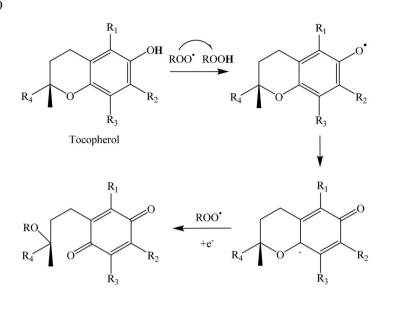


Fig. 3. General protein oxidation mechanism

PH: protein, •OH: hydroxyl radical, P•: protein radical, H<sub>2</sub>O: water, O<sub>2</sub>: oxygen, POO•: analkyl proxyl radical, PO•: alkoxyl radical, POOH: alkyl peroxide,  $M^{n+}$ : reduced forms of transition metals,  $OH^{-1}$ : hydroxide anion, POH: hydroxyl derivative, HO<sub>2</sub>: hydroperoxyl radical, an H<sup>+</sup>: hydron.

A)



B)

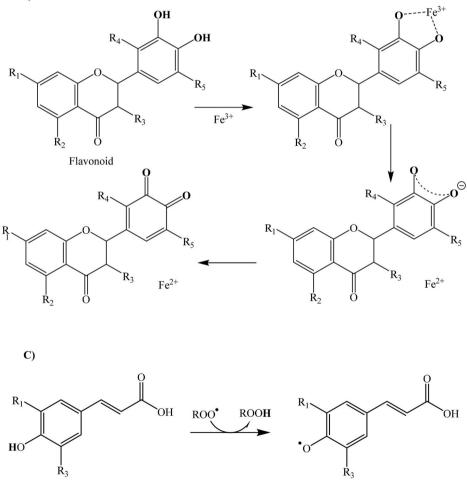




Fig. 4. Mechanism of action of some natural antioxidants: A) Tocopherol scavenging a free radical by donating a hydrogen atom. B) Flavonoid chelating a metal ion. C) Phenolic acid (derived from cinnamic acid) scavenging a free radical by donating a hydrogen atom.

interaction between malondialdehyde (MDA) and thiobarbituric acid (TBA) that leads to the formation of pink-colored products that are absorbed at 532 nm. MDA is formed as a result of lipid oxidation and is a reactive electrophilic species that causes toxicity in the cells. This assay takes advantage of the production of MDA to follow lipid oxidation. It is worth noting that MDA is not exclusive of lipid oxidation. In other words, this test is not completely specific; it is, generally, accepted by the scientific community, instead. Using visible spectrometry and comparing measurements when no antioxidants are added or when antioxidants are added, it is possible to elucidate the ability of a compound to stop the oxidation of a food, given that its activity interferes with the formation of pink-colored products (Gülçin, 2012; Manessis et al., 2020).

The DPPH assay is also used for determination of antioxidant capacity. DPPH is a nitrogen-based, stable, purple-hued radical that can be reduced by antioxidant compounds to a yellowish counterpart, known as diphenyl-picrylhydrazine. When this happens, a decrease in the previous coloration can be observed and a follow-up can be carried out by absorbance. Maximum absorbance of DPPH occurs at 517 nm; when the antioxidants reduce this compound, a drop in absorbance is observed (Gülcin, 2012). Another method for this task is the Ferric Reducing Antioxidant Power (FRAP). In this case, the ability of the antioxidant compounds to reduce a complex of  $[Fe^{3+}-(TPTZ)_2]^{3+}$ to  $[Fe^{2+}-(TPTZ)_2]^{2+}$  at low pH (for iron to remain soluble) is measured (Guyon et al., 2016). This causes the formation of ferrous-trypidyltriazine, which is a compound that has a characteristic blue color. FRAP values are taken by measuring the absorbance at 593 nm. The stronger is the intensity of the color, the greater will be the amount of  $\mathrm{Fe}^{2+},$  and therefore, the higher the antioxidant capacity of the tested compound. This assay also has its disadvantages; for example, not all antioxidant compounds reduce Fe<sup>3+</sup> fast enough to be considered in the measured values. In addition, any compound with a lower reduction potential than Fe<sup>3+</sup>/Fe<sup>2+</sup> could reduce Fe<sup>3+</sup>, contributing to the measurements made (Gülcin, 2012). The ABTS test measures the antioxidant neutralise capacity the stable 2.2' to -azinobis (3ethylbenzthiazolin-6-sulfonic acid) radical cation (ABTS<sup>•+</sup>) stable radical cation, a blue-green chromophore of maximum absorption at 734 nm, whose intensity decreases in the presence of antioxidants. ABTS<sup>•+</sup> may be generated from ABTS in the presence of powerful antioxidant agents. The degree of discolouration of the blue-green color, quantified as a sudden drop in absorbance to 734 nm, depends on the reaction duration, intrinsic antioxidant activity, and sample concentration. The oxygen radical absorption capacity (ORAC) test measures the splitting ability of the radical chain reaction by antioxidants through monitoring the inhibition of the oxidation of the peroxyl radical. Peroxyl radicals are characterised as free radicals that predominate in lipid oxidation in biological systems and also in foodstuffs, under physiological conditions. As a result, ORAC values are appreciated by certain researchers as biologically relevant, a benchmark for antioxidant efficiency (Munteanu & Apetrei, 2021).

It is also important to study the behaviour of antioxidants not only when they are applied directly to the meat, but also when the meat has a large number of emulsions. Oil and water in meat structure which can form an oil-in-water emulsion, wherein the oil is the dispersed phase, and water is the dispersion medium. It is at the water/oil interphase that lipid oxidation begins in this type of system. The double bonds present in the structure of unsaturated fatty acids in emulsions are very susceptible to oxidation. Normally, a single type of antioxidant does not provide complete protection to emulsified systems since there are different mechanisms for their oxidation. In fact, the antioxidants can have more affinity for one phase of the system than for the another (Zhang et al., 2021).

Characteristics to take into account about these antioxidants to be able to use them in these cases range from the size of the molecule to its mechanism of action and behaviour with respect to the emulsifying agent, with the polarity of the antioxidant molecule being the most important characteristic (Di Mattia et al., 2010). It also has been shown that is possible to increase the defense capacity of antioxidants present in emulsified systems against lipid oxidation easily by varying their position in the system. Studies carried out with gallic acid showed that although this phenolic acid has a higher affinity for the aqueous phase due to its high polarity and therefore its distribution at the interphase is not the same, it is effective in stopping lipid oxidation in emulsified systems. For quercetin, the distribution in the two phases is more balanced, and a reduction in lipid oxidation has been demonstrated, due to its lower polarity. Both, gallic acid and quercetin have also proved the ability to reduce surface tension in the emulsions (Kiokias & Varzakas, 2017). Another similar study shows that the combination of tocopherol and catechol, hydrophobic and hydrophilic compounds respectively, was able to protect most of the fatty acids in an emulsion. Thus, it can be stated that by using a combination of antioxidants better protection performances can be obtained than by using a single type of antioxidants.

## 4.3. Prevention of protein oxidation

There are a few methods for determination of protein oxidation, too, which are based on the detection of protein oxidation products, or detecting the modifications that may occur in amino acids (e.g., determination of thiol group losses). When this oxidation process occurs, the cysteine and methionine of an amino acid chain in certain proteins lose thiol group by the degradation caused by oxidizing compounds. Therefore, thiol group losses can be measured to keep track of this phenomenon; this is achievable using DTNB (5,5'-dithiobis(2-nitrobenzoate)) that binds to the free thiol groups releasing a thiolate ion, which is colored and has an absorption maximum at 412 nm (Nollet & Toldrá, 2009). Another most used way to follow this oxidative process is determination of carbonyl compounds produced in large quantities as a result of protein degradation. The method consists of reacting the carbonyl compounds with DNPH (2,4-dinitrophenylhidrazine) to form 2,4-dinitrophenylhidrazone, which can be followed by spectrometry at 370 nm. On the other hand, the protein level is measured without the addition of DNPH for comparison at 280 nm (Nollet & Toldrá, 2009).

Some of the above methods are widely used in the literature for determining the antioxidant capacity of certain plant extracts, in the hope that these natural by-products will have the same effectiveness in preserving and prolonging the shelf-life of meat products compared to the old compounds used for the same task. Table 1 shows a selection of studies in which different natural by-products have been tested, the quantity of these products used for the study, the type of meat product for which they were used, the storage to which the products were subjected, and the effects they had on the oxidative processes of both lipids and proteins. Most of the studies do not present data on protein oxidation because lipid oxidation is much more influential in the conservation of the meat products, and it is a new subject that has begun to be addressed just recently.

Previous studies have, generally, dealt with the treatment of meat products with the extracts of slaughtered and butchered animal byproducts, but it is also possible, as was mentioned very briefly earlier in this section, to feed the animals a diet rich in antioxidants (Salami et al., 2016). This review is not focused on this kind of studies and therefore will not go into further detail on the subject; however, it is good to indicate that the possibility exists and can be the subject of future studies.

# 5. Encapsulation of plant by-product antioxidants for meat and meat products

Several bioactive compounds isolated from botanical sources cannot be simply used directly due to their low solubility in water, low chemical stability, or limited biological activity (Hadidi, Rostamabadi, et al., 2022). To provide the best possible dispersibility, chemical stability and

# Table 1

Natural antioxidants derived from by-products to control oxidation in meat and meat products.

| Plant by-  | Major compounds  | Quantity<br>added<br>0.075–0.150<br>µL/g | Meat product<br>Ground pork<br>patties | Storage<br>condition<br>4 °C for 3 days  | Oxidation in meat and meat   | Reference  |                                   |  |
|--|--|--|--|--|--|--|-----------------------------------|--|
| product  |  |  |  |  | Lipid oxidation  | Protein oxidation  |                                   |  |
| Wild thyme by-<br>product<br>extract   | Carvacrol, thymol and $\alpha$ -terpineol  |  |  |  | Addition of extracts<br>inhibited lipid oxidation<br>(TBARS value) during  | Protein oxidation (thiol<br>content) was decreased<br>significantly during   | Šojić et al. (2020)               |  |
| Rice bran<br>extract   | p-coumaric acid<br>ferulic acid,<br>γ-oryzanol                                     | 5-20 gr                                  | Pork burgers                           | Cold storage at<br>4 °C for 21 days  | storage.<br>The extract-treated<br>burgers maintained their<br>initial oxidation values<br>(TBARS value) compared<br>to the controls, where<br>oxidation values increased.   | storage.<br>Carbonyl concentration<br>was inversely proportional<br>to the amount of added<br>extract.   | Martillanes et al.<br>(2020)      |  |
| Grape seed<br>extract with<br>olive pomace<br>and chestnut<br>extract with<br>olive pomace | Hydroxytyrosol and<br>tocopherol   | 10 gr/kg                                 | Dry-<br>fermented<br>pork<br>sausages  | Vacuum-packed<br>and –80 °C<br>storage   | The two mixtures can act<br>as a substitute for nitrites<br>in lipid oxidation.  | -  | Aquilani et al.<br>(2018)         |  |
| Blueberry<br>pomace  | Phenolic acids,<br>flavonoids, and<br>anthocyanins                                 | 1–2% (w/w)                               | Pork burgers                           | Refrigerated<br>storage (3–5 °C)<br>without light for<br>7 days                | Delays lipid oxidation<br>through the reduction of<br>volatile oxidizing<br>compounds present thanks<br>to the scavenging effect of<br>phenols in the by-product.  | -  | Peiretti et al.<br>(2020)         |  |
| Olive waste<br>extract   | Tyrosol and<br>hydroxytyrosol  | 100-400 mg/<br>kg                        | Lamb<br>burgers                        | -20 °C storage<br>for 0-days<br>samples. Dark<br>storage at 4 °C<br>for 9-days | After 2 days of storage, the<br>TBARS value of the control<br>group increased<br>considerably compared to<br>the treated groups.   | The control group showed<br>a higher percentage of<br>protein carbonylation than<br>the treated groups. The<br>higher was the amount of<br>extract, the less protein<br>carbonylation was<br>observed.   | Muíño et al.<br>(2017)            |  |
| Olive mill<br>extract  | Hydroxytyrosol,<br>tyrosol, and<br>verbascoside                                    | 75–150 mg                                | Pork<br>sausages                       | Freezing with<br>liquid nitrogen<br>and storage at<br>-80 °C 14-days           | The amount of TBARS,<br>cholesterol oxidation<br>products and peroxide<br>values increased in the<br>treated raw products with<br>storage time, but their<br>values were lower than<br>those of the control group.   | -  | Balzan et al.<br>(2017)           |  |
| Pistachio green<br>hull water<br>extract   | Gallic acid and<br>protocatechuic acid   | 250–1000<br>mg/kg                        | Beef burgers                           | Refrigerated<br>storage at 4 °C<br>for 8 days                                  | Values of TBARS and<br>peroxides were measured<br>in the groups treated with<br>different concentrations of<br>extract compared to the<br>control group. No decrease<br>in DPPH levels was<br>observed compared to the<br>control group.                         | The DNPH assay was used<br>to follow protein oxidation<br>through the formations of<br>carbonyl groups.<br>Compared with the<br>control, the groups treated<br>with different<br>concentrations of extract<br>showed lower values of<br>carbonyls. | Sadeghinejad<br>et al. (2019)     |  |
| Mix of extracts<br>of green tea,<br>stinging,<br>olive and<br>nettle leaves                | Catechin,<br>epigallocatechin,<br>and epigallocatechin<br>gallate                  | 210 g/kg                                 | Frankfurter-<br>type sausage           | Stored at 4 °C in<br>vacuum packs<br>for 45 days                               | The extracts provided a<br>high number of<br>polyphenolic compounds<br>that reduced the lipid<br>oxidation. The number of<br>total nitrogen compounds<br>and TBARS in the treated<br>samples was lower than in<br>the controls during the 45<br>days of storage. | _  | Alirezalu et al.<br>(2019)        |  |
| Olive tree<br>vegetation<br>water extract<br>and rosemary<br>extract                       | Hydroxytyrosol   | 200 ppm                                  | Lamb burger                            | Refrigerated<br>stored at 4 °C in<br>aerobic<br>conditions for 6<br>days       | Both extracts showed good<br>preservation capabilities<br>(TBARS content)<br>compared to the control<br>group (In this case, the<br>control group was treated<br>with sulfites and other<br>synthetic antioxidants).   | Protein oxidation was<br>determined on the basis of<br>thiol concentration.<br>It was observed that the<br>two extracts acted as pro-<br>oxidants of thiol groups.   | (Martínez-zamora<br>et al., 2020) |  |
| Sweet basil<br>essential oil   | Estragole, 1, 6-octa-<br>dien-3-ol, 3,7-<br>dimethyl, and<br>$\alpha$ -bergamotene | 2–6% (w/w)                               | Minced beef                            | Refrigerated<br>stored at 4 °C for<br>7 days                                   | The addition of 2–4% of<br>essential oil reduced<br>TBARS values within 0–4<br>days of storage. After 7<br>days of storage, the<br>samples treated with 4–6%<br>of essential oil showed  | -  | Falowo et al.<br>(2019)           |  |

(continued on next page)

#### Table 1 (continued)

| Plant by-<br>product      | Major compounds | Quantity<br>added | Meat product       | Storage condition                   | Oxidation in meat and meat  | Reference         |                         |
|---------------------------|-----------------|-------------------|--------------------|-------------------------------------|---|-------------------|-------------------------|
|                           |                 |                   |                    |                                     | Lipid oxidation   | Protein oxidation |                         |
| Moringa flower<br>extract | Phenolics       | 1–2% (w/w)        | Chicken<br>nuggets | Cold storage at<br>4 °C for 20 days | TBARS values higher than<br>those in the control group.<br>The TBARS values for the<br>control group were higher<br>with the passage of storage<br>time as compared to those<br>treated with the extract. At<br>15 days of storage the<br>extract-treated groups<br>remained at acceptable<br>values. | -                 | Madane et al.<br>(2019) |

matrix compatibility, it is important to encapsulate these components within specially designed colloidal particles (Domínguez et al., 2020). Encapsulation is one of the most innovative methods for enhancing the stability and function of meat products through the addition of

antioxidant compounds; this preserves natural antioxidants by encasing them in one or more wall materials and allowing them to avoid direct contact with food components. Increasing the efficacy food components through encapsulation has also been employed in a variety of ways to

Table 2

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Effect of encapsulated plant by-product antioxidants for control of protein-lipid oxidation in meat and meat products.
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| Plant by-<br>product<br>sources                         | Major<br>compounds   | Encapsulation<br>method                      | Wall material                                     | Concentration        | Meat<br>product          | Effects on protein-lipid oxidation   | Storage condition             | Ref.                              |
|---|--|--|---|----------------------|--------------------------|--|-------------------------------|-----------------------------------|
| Olive leaf<br>extract                                   | -  | Complex<br>coacervation or<br>lyophilization | Gelatin and<br>tragacanth<br>gum                  | 0.57 g/56 g          | Sheep meat<br>hamburgers | A significant increase<br>was observed in the<br>peroxide and TBARS<br>indexes of hamburgers,<br>Encapsulation increased<br>antioxidant activity of<br>the extract                       | At -15 °C<br>for 4<br>months  | Oliveira et al.<br>(2022)         |
| Propolis co-<br>product<br>extract                      | Phenolic<br>compounds<br>(coumaric acid,<br>epicatechin and<br>caffeic acid) | Spray drying                                 | Modified<br>starch                                | 0.3 g/kg             | Burger meat              | Encapsulation enhanced<br>the TBARS values of<br>extract during the entire<br>storage period.  | At 15 °C<br>for 28<br>days    | Reis et al. (2017)                |
| Pomegranate<br>peel                                     | Phenolic<br>compounds<br>(punicalagin,<br>chyrsin and<br>ferulic acids)      | Freeze drying                                | Bovine gelatin<br>type B and<br>tragacanth<br>gum | 1 and 1.5%           | Minced beef<br>meat      | Retarding lipid and<br>protein oxidation in<br>meatballs.<br>Encapsulation by bovine<br>gelatin and tragacanth<br>gum as wall materials<br>enhanced both protein<br>and lipid oxidations | At 4 °C for<br>15 days        | Morsy et al.<br>(2018)            |
| Avocado peel<br>extract                                 | Flavonoids and other phenolics   | Complex<br>coacervation                      | Collagen and pectin                               | 0.28 g/200 g<br>meat | Ground beef              | Reduction in the<br>oxidation of proteins of<br>approximately 45% by<br>application of collagen<br>and pectin as wall<br>materials   | At 4 °C for<br>10 days        | (Calderón-Oliver<br>et al., 2020) |
| Bay ( <i>Laurus</i><br><i>nobilis</i> ) leaf<br>extract | Flavonoids and other phenolics   | Nanoencapsulation<br>with liposome           | Lecithin  | 1000–1500<br>ppm     | Minced beef              | TBA and PV value was<br>increased in all<br>treatments during the<br>storage period.<br>Encapsulation had a<br>significant effect on<br>enhancement of<br>antioxidant activity           | At 4 ±<br>1 °C for<br>16 days | Tometri et al.<br>(2020)          |
| Pitaya<br>(Hylocereus<br>costaricensis)<br>peel         | -  | Spray drying                                 | Maltodextrin                                      | 100 and 1000<br>ppm  | Ground pork<br>patties   | Delayed the protein<br>oxidative process by<br>encapsulation of extract<br>into maltodextrin<br>nanoparticles  | At 4 °C for<br>9 days         | Cunha et al.<br>(2018)            |
| Jabuticaba<br>(Myrciaria<br>cauliflora)<br>extract      | Phenolic<br>compounds and<br>anthocyanins                                    | Spray dryer                                  | Maltodextrin                                      | 2% and 4%            | Fresh<br>sausage         | TBARS values were<br>lower throughout the<br>storage period  | At 1 °C for<br>15 days        | Baldin et al.<br>(2016)           |
| Pineapple Peel  | Phenolics  | Spray drying                                 | Maltodextrin                                      | 0.3%                 | Beef meat                | Retarded lipid oxidation<br>in meat samples. Lipid<br>oxidation stability of<br>was improved after<br>encapsulated in<br>maltodextrin  | At 4 °C for<br>5 days         | Lourenço et al.<br>(2020)         |

overcome some of their limitations and boost their bioavailability and solubility (Gómez et al., 2018; Hesami et al., 2021). Some of these encapsulation-based techniques of natural antioxidants include freeze drying, spray-drying, coacervation, etc. (Hadidi et al., 2021). There have been several studies on the encapsulation of plant extract components for use as functional ingredients within the food matrix and in the formulation of food coatings, edible films, and active packaging materials (Hadidi, Rostamabadi, et al., 2022). The US Food and Drug Administration (FDA) has recognized bioactive compounds used in such materials as generally recognized as safe (GRAS). As shown in Table 2, various plant materials, including roots, peels, pulps, pods, berries/fruits, leaves, seeds, or agro-food by-products are potential sources of these essential bioactive compounds that could be employed as natural and promising additives in meat production (Umaraw et al., 2020).

García-Lomillo et al. (2014) investigated a variety of powdered extracts made from grape waste. They discovered that all of the extracts showed antioxidant capability with seeds having the highest potential, followed by pomace and skins. This ability is attributed to the presence of anthocyanins with a strong ability to scavenge reactive radicals produced by fat oxidation, according to the scientists. Moreover, it was discovered that adding encapsulated Laurus nobilis leaf extract to minced beef via nanoliposomes protected it from deterioration. The extract also protected the meat against other significant degradative events, including lipolysis in free fatty acids (FFAs) and proteolysis (measured by total volatile basic nitrogen). Moreover, it protected the meat against other significant degradative events like lipolysis (measured in FFAs) and proteolysis (measured by total volatile basic nitrogen). Lipolytic and proteolytic processes are widely recognized to be linked to the activity of microbial enzymes. Therefore, the above extract's antibacterial activity was linked to the decrease in the lipolytic and proteolytic processes. The inclusion of encapsulated and non-encapsulated extracts was also demonstrated to have a significant inhibitory effect on E. coli and S. aureus growth, as well as a reduction in total viable and psychrotrophic bacteria counts (Tometri et al., 2020).

Olive leaf extract has been shown to have the ability to function as a natural antioxidant in meat products, avoiding oxidation. Oleuropein is the most prevalent phenolic component responsible for the bitter flavor of olives and olive oil. Verbacoside (11.1%), luteolin-7-glycoside (1.4%), apigenin-7-glycoside (1.4%), and hydroxytyrosol (1.5%) were other polyphenols found in the highest concentrations in this extract (Oliveira et al., 2022). Accordingly, some authors are on the belief that avocado peel extract has antioxidant properties due to its high polyphenol content (Calderón-Oliver et al., 2017). However, some preservatives such as polyphenols in food might cause sensory alterations, interact with other compounds (lowering their antioxidant activity), or be destroyed by proteases, as in the case of nisin in meat (Fang & Bhandari, 2010). Epicatechin, isorhamnetin and kaempferide epicatechin gallate, widely found in avocado peel extract (approximately 19.71 mg equivalents of gallic acid/g of extract), are reportedly able to prevent or reduce oxidation in minced meat (Calderón-Oliver et al., 2020).

#### 6. Conclusions

Protein-lipid oxidation reactions are among the most common causes of quality deterioration in meat and meat products. Natural antioxidants derived from plant by-products are increasingly being used to control oxidative alterations in muscle food during storage. Protection against protein-lipid oxidation, discoloration, and microbial growth extends the shelf-life of meat products, which is so important for both customers and producers. Due to the high-cost and negative health effects such as carcinogenic and toxicological consequences of many synthetic antioxidants, the meat industry is demanding natural source basedantioxidants to substitute synthetic antioxidants. Plant by-products as novel alternative sources of low-cost natural antioxidants, apart from oxidation inhibition, may also affect other quality attributes of foods, especially meat and mead products. It is worth mentioning that more study is being performed by researchers on the application of natural antioxidants derived from plant by-products to further confirm their safety for human health.

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#### CRediT authorship contribution statement

Milad Hadidi: contributed to conceptualization, review the final draft and supervision. Jose C. Orellana-Palacios: contributed to writing of initial manuscript and review the final draft. Fatemeh Aghababaei: contributed to review the final draft. Diego J. Gonzalez-Serrano: contributed to review the final draft. Andres Moreno: contributed to review the final draft. Jose M. Lorenzo: contributed to conceptualization and review the final draft.

# Declaration of competing interest

All authors declare no conflict of interest regarding this investigation.

## Data availability

No data was used for the research described in the article.

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