Critical overview of the use of plant antioxidants in the meat industry: Opportunities, innovative applications and future perspectives

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ABSTRACT
The number of articles devoted to study the effect of “natural antioxidants” on meat systems has remarkably increased in the last 10 years. Yet, a critical review of literature reveals recurrent flaws in regards to the rationale of the application, the experimental design, the characterisation of the plant sources, the discussion of the molecular mechanisms and of the potential benefits. The selection of the appropriate source of these antioxidants and the identification of their bioactive constituents, are essential to understand their mode of action and set effective and safe doses. The methodological approach should also be planned with care as the recorded effects and main conclusions largely depend on the accuracy and specificity of the methods. This article aims to critically review the recent advances in the application of plant antioxidants in meat and meat products and briefly covers current trends of innovative application and future trends.

1. Introduction

Lipid oxidation continues to be a challenge to the food industry (Domínguez, Pateiro, Gagaoua, et al., 2019; Kalogianni, Lazou, Bossis, & Gelasakis, 2020). Food lipids are continuously exposed to endogenous and environmental pro-oxidants, which leads to the degradation of fatty acids and the generation of off-flavors and potentially toxic compounds (Domínguez et al., 2019). Meat systems are particularly susceptible to lipid oxidation owing to the occurrence of pro-oxidants among muscle components (i.e. heme iron) and the application of assorted technologies that promote oxygen exposure (i.e. size reduction) and formation of reactive oxygen species (heating, salting, high-pressure, irradiation…) (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013). Furthermore, the limited endogenous antioxidant defences in muscle tissue partially collapse after slaughter, facilitating the onset of oxidative reactions during the subsequent aging, processing, and storage (Bekhit et al., 2013). The perception of rancidity and off-flavors, along with the remarkable loss of essential nutrients (fatty acids and vitamins), are major consequences of the oxidative degradation of unsaturated fatty acids (UFA) in processed meat products (Domínguez et al., 2019). In fresh unprocessed meat, the oxidation of heme iron and the accumulation of metmyoglobin remains the main cause of quality loss and consumer rejection, leaving, unquestionably, microbial spoilage aside (Faustman, Sun, Mancini, & Suman, 2010). The application of antioxidant strategies seems to be justified by the loss of economic loss in the meat industry owing to consumer rejection (Falowo, Fayemi, & Muchenje, 2014). Moreover, the scientific evidence of the health impact caused by the intake of oxidized lipids provides further motivations to control the accumulation of malondialdehyde and other toxic species in oxidized meat products (Vieira, Zhang, & Decker, 2017). Along with heme iron and lipids, meat proteins are also sensitive to the pro-oxidant action of reactive oxygen species (ROS) and reducing sugars (glycoxidation) (Luna & Estévez, 2018, 2019; Soladoye, Juarez, Aalhus, Shand, & Estévez, 2015). Protein oxidation has also gained considerable interest in recent years given the proven impact of oxidized proteins on meat quality traits such as tenderness and water-holding capacity (WHC) (Soladoye et al., 2015; Zhang, Xiao, & Ahn, 2013). In addition to the depletion of essential amino acids and the subsequent nutritional loss (Soladoye et al., 2015), dietary oxidized amino acids such as dityrosines and the α-amino adipic acid (α-AA), among others, have also been found to impair physiological processes in cultured human cells (Díaz-Velasco, González, Peña, & Estévez, 2020; Estaras, Ameur, Estévez, Díaz-Velasco, & Gonzalez, 2020) and murine models (Ge, Li, Yang, et al., 2021; Li, Shi, Le, Ding, & Zhao, 2016). The connection between the intake of oxidized meat and meat products and human health has been profusely documented (Estévez, Li, Soladoye, & Van-Hecke, 2017; Estévez & Luna, 2017; Estévez & Xiong, 2019; Macho-González, Garcimartín, López-Oliva, et al., 2020) and emphasizes the necessity of antioxidant solutions for the meat industry.

Driven by the impact of food and certain dietary patterns on the
onset of chronic diseases (Kopp, 2019), consumers generally show a clear preference for foods generally perceived as healthy and, in this context, plants and vegetables have attracted considerable attention (Boeing, Bechthold, Bub, et al., 2012). In this scenario, much of the recent research in the field of redox reactions in meat systems, has been driven by the interest in identifying sources of antioxidant compounds in the plant kingdom and assess their protective effect on meat systems (Pateiro, Gómez-Salazar, Jaime-Patlan, Sosa-Morales, & Lorenzno, 2021). The profuse literature in the application of the so-called “natural anti-
oxidants” covers a wide range of plant sources, meat systems and application modes and doses. This enormous variability hinders a straightforward comparison of effectiveness among plant bioactives. Furthermore, the methods employed to assess the protection of the plant materials against meat oxidation are numerous, and at times, limited in their appropriateness to understand the action mechanisms of the bioactive compounds. The studies aimed to assess the effectiveness of “natural antioxidants” in meat products have evolved from relatively simple “cook-and-look” type of experiments to highly sophisticated mechanistic assays. In the former, a given plant material, or its extract is added to a given meat product to check whether the treatment leads to a reduction in thiobarbituric acid-reactive substances (TBARS) or protein carbonyls (Estévez & Cava, 2006; Ganbhío, Estévez, Armenteros, & Morcuende, 2013). The lack of a rationale in the selection of materials, initial hypothesis, and proper design and methodological approach, leads to results of poor scientific in-depth and hazardous industrial application. The unawareness of the nature and concentration of bioactive compounds and the molecular basis of their functionality leads to uncertainty, inconsistent outcomes and serious concerns of their safety. Conversely, studies aimed to provide insight into mechanisms and antioxidant capacities of definite chemical species or well-characterized plant extracts deliver scientific knowledge that enable consistent and safe practical applications (Bolumar, Andersen, & Orljen, 2014; Iglesias, Pazos, Torres, & Medina, 2012). The abundant and at times, chaotic information available in the literature about the application of natural antioxidants in meat products, calls for a concise critical review of the current experimental plans and methodological methods and a description of potential future directions in this field.

2. Use of plant antioxidants in meat products: Rationale

2.1. Arguable motivations

The description of the assorted and serious consequences of the onset of oxidative reactions in meat and meat products seems to justify the application of antioxidant solutions and, hence, inhibit or alleviate the impact of such reactions on meat quality. Yet, the occurrence of oxida-
tive reactions, their severity and consequences largely vary between meats and meat products (Bekhit et al., 2013; Soladoye et al., 2015). This context, it seems essential to perform an initial risk assessment of oxidation as a relevant source of quality or safety decline and therefore, consider if the time and effort required to develop and apply an anti-
oxidant strategy is needed. On this line, it is essential to identify the potential threat caused by the onset of the oxidative reactions in a particular meat system and envisage the potential benefits that such antioxidant strategy should eventually display. The definition of a reasonable initial hypothesis guarantees the rationale behind the study and facilitate the setup of an appropriate and effective experimental design, which may include treatments, replications, selection of the antioxidant source and means of application.

On this line, the assessment of lipid/protein oxidation in fresh/un-
cooked meat as a marker of quality deterioration is a matter of discus-
sion. It is generally recognized that meat shelf life is mainly limited by microbial spoilage (Doulgeraki, Ercolini, Villani, & Nychas, 2012), and besides that, discoloration is the main abiotic threat to meat quality (Purslow, Warner, Clarke, & Hughes, 2020). The analysis of heme iron oxidation, accumulation of metmyoglobin and color evolution during aging/storage seem appropriate means to assess the direct impact of oxidative reactions on fresh meat quality (Suman & Joseph, 2013). Conversely, the accretion of TBARS or proteins carbonyls in these samples generally provide information of poor practical application. The severity of oxidative changes suffered by fresh meat during subsequent culinary preparation or technological processing is such (Bekhit et al., 2013; Soladoye et al., 2015), that preceding mild oxidative reactions may have little impact on eating quality traits. However, assessing lipid/protein oxidation in fresh meat may be relevant if complementary an-
alyses are performed to signify the impact of such reactions in terms of nutritional value, sensory properties, or safety. For instance, thiol oxidation and crosslinks formation has been found to impact tenderness during meat aging (Lund, Lametsch, Hvid, Jensen, & Skibsted, 2007; Zhang, Cheng, Wang, & Fu, 2020; Zhang, He, Li, et al., 2020). Under-
lying mechanisms by which protein carboxylation contribute to impair WHC in fresh meat have also been reported (Bao, Boeren, & Ertbjerg, 2018; Utter & Estévez, 2012). Similarly, the analysis of lipid oxidation in aged meat provides relevant information on the mechanisms behind the transformation of muscle into meat, on the understanding of redox balance in post-mortem meat and the contribution of lipid-derived degradation products during aging on flavor-active compounds and colour deterioration (Campo, Nute, Hughes, et al., 2006; Rant, Radzik-
Rant, Swiatek, et al., 2019). In any of these exemplary scenarios, the introduction of redox-active compounds is justified to protect meat against oxidation-driven texture deterioration, discoloration or provide further insight on how dietary or added antioxidants could modulate redox reactions during aging and hence, improve meat quality and safety. The sole reduction of TBARS or protein carbonyls in fresh meat, as a result of an antioxidant manipulation, provides null information of scientific of practical relevance.

Once the necessity of an antioxidant strategy is justified, the selec-
tion of a “natural antioxidant” as the suitable choice should also be explained. A detailed analysis of the available literature reveals some justification templates on the use of antioxidants from plant kingdom and occasionally, the arguments rendered are doubtful and misleading. Some of these arguments are recurrently used in an aixom fashion though the scientific basis is weak. One main argument behind the use of “natural antioxidants” is the necessity of replacing “synthetic anti-
oxidants” owing to the safety and health concerns of the latter (Estévez, Ventanas, & Cava, 2006; Naveena, Sen, Vaithiyanathan, Babji, & Kondaiah, 2008; Sebranek, Sewalt, Robbins, & Houser, 2005). Once the objection on the synthetic is exposed, the justification of the application of “natural antioxidants” as appropriate alternatives is supported by their alleged effectiveness and safety. Emphasizing the safety of plant materials and their extracts because of their “natural” occurrence in plant kingdom is an unfortunate and daring argument with no scientific basis. This is particularly regrettable when this argument is unfounded for an unusual source of “natural antioxidants” and no scientific evidence of the safety of such specific plant material, is reported (Cando, Morcuende, Utterra, & Estévez, 2014). Authors should be particularly cautious when using pure plant chemical species or concentrated iso-
lates because their bioactive effects could be, depending on the dose (among other many factors), negative in terms of meat quality (Cando et al., 2014; Jongberg, Terkelsen, Miklos, & Lund, 2014) and noxious to the final consumer (Castaneda-Arriaga, Pérez-Gonzalez, Reina, Alvarez
dadoy, & Galano, 2018; Kyselova, 2011). On this line, it is worth mentioning that the synthetic antioxidants typically reported to display safety concerns (BHA, BHT and others) are safe at the doses and appli-
cations allowed by the corresponding legal authority (EFSA in Europe and FDA in USA, as examples). Unlike most of those chemical species identified as “natural antioxidants”, synthetic antioxidants authorised as food additives have positively passed through demanding safety studies. This safety assessment is re-evaluated and whenever a new evidence questions the safety of particular antioxidant additives, their entries as food additives are removed. The recent decision of the European Com-
mision (EC) to prohibit the use of octyl gallate (OG) and dodecyl gallate
used as antioxidants in specific meat products by the Food and Drug Administration (FDA) of the US (CFR, 2020). Secondarily, the aforementioned synthetic antioxidants are highly lipophilic compounds, and their action is mostly expected in the lipid fraction. Chemicals from plant extracts display a wide range of polarity but most of them act in the interphases (tocopherols and some polyphenols) or in the polar phase of emulsions or comminate meat products (ascorbate, most phenolic acids, flavonoids and some polyphenols). Therefore, the divergent lipid-water partition of “natural” and synthetic antioxidants suggest that their actions would be different and likely complementary when added to a given meat product.

Other recent practise in the use of plant materials in meat products aims to replace some additives which, despite of being effective and safe at the allowed doses, may raise some distrust among consumers. Nitrates and nitrates are involved in the formation of nitrosamines and other harmful N-nitroso compounds (Demeyer & De Smet, 2010) and therefore, its usage as curing agents has been recurrently objected (Alahakoon, Jayasena, Ramachandra, & Jo, 2015). Assessing the suitability of a plant material, rich in versatile bioactive compounds, to replace the antioxidant and antimicrobial effects of nitrite, is a topic of scientific and technological interest (Alahakoon et al., 2015; Cui, Gabriel, & Nakano, 2010). This logical approach loses its credibility when the replacement of added nitrite (or nitrate) is made by the inclusion of green-leaved plants in which nitrate naturally occurs at high concentrations (Santamaria, 2006). Labelling these products as “no nitrate or nitrite added” and/or “uncured” leads to consumer’s misleading perception and has risen a fiery controversy as recently reviewed by Flores and Toldrá (2021). Suggesting that meat products cured with nitrate/nitrite naturally present in plants, are safer or healthier, than those cured with the chemically identical nitrate included as an additive (Jo, Lee, Young, Choi, & Jung, 2020; Shin, Hwang, Lee, et al., 2017) is an act of scientific rigor disregard. As recently emphasized by Rivera, Bunning, and Martin (2019), consumers may concurrently denigrate conventional curing agents and willingly accept the natural forms of the same constituents. Yet, the chemical and safety implications of nitrate/nitrate in meat products, regardless of their origin (synthetic or natural), persevere. While some economic bodies may overtly distort scientific knowledge and deliver deceitful claims on the benefits of “natural” nitrate over “synthetic” nitrate, meat scientist should avoid the alignment with such unfortunate marketing strategies. Nevertheless, scientific literature is profuse with pertinent studies aimed to take advantage of the multiple bioactive components of fruits and vegetables (i.e. dietary fibre, phenolics, minerals, vitamins, among others) to design processed meat products with enhanced nutritional and safety properties. In this regard, togarono and other herbs and spices have been found to display antioxidant activity (Cui et al., 2010), tomato pulp has been tested as preservative in pork luncheon with reduced nitrite concentration (Hayes, Canonico, & Allen, 2013), and citrus by-products has been included in cured meat products to decrease the residual nitrite level (Fernández-Gínés, Fernández-López, Sayas-Barberá, Sendra, & Pérez-Alvarez, 2003; Viuda-Martos, Fernández-López, Sayas-Barbera, et al., 2009). This is very much in line with the strategies aimed to produce “clean label” foods, which will be briefly covered in due course.

2.2. Current opportunities

The meat industry is in need of effective and safe antioxidant solutions and antioxidants derived from plant kingdom can be excellent options. It is essential, however, to identify the convenient combination of target meat product and appropriate plant-based source of bioactive compounds. In the regards to the former, a number of meat and meat products are recognized to be particularly susceptible to lipid and protein oxidation and hence, the addition of plant-derived antioxidants may be required. This is particularly relevant when such meat products are designed to contain reduced levels of additives with proven antioxidant activity (nitrite, phosphates, among others). Such replacement may require a comprehensive analysis of the technological and safety hazards that such replacement may involve. Assorted plant materials have been tested in genuine nitrite-free or low-nitrite meat products to replace the antioxidant effect of this curing agent in cured muscle foods. Vossen, Urrera, De Smet, Morcuende, and Estévez (2012) employed dog rose (Rosa canina L.) as a source of antioxidant phytochemicals in porcine frankfurters without added sodium nitrite. Aquilani, Sirtori, Flores, et al. (2018) studied the effect of antioxidants from grape seed and chestnut in combination with hydroxtyrosol, as sodium nitrite substitutes in Cinta Senese dry-fermented sausages. Sabin, Samli, Biretks, Tan, et al. (2017) evaluated the antioxidant and antimicrobial properties of polyphenols from olive tree leaves for their potential as substitutes of conventional curing agents. Alirezalou, Hesari, Nemati, et al. (2019) assessed the antioxidant capacity of betacarmins as indole derived plant pigments, such as betanin, phyllocactin and betanidin in nitrite-free frankfurters. More recently, Karim, Fathi, and Soleimanian-Zad (2020) applied nanoencapsulated cinnamaldehyde to cooked sausages as a strategy to reduce nitrite levels. The authors observed bactericidal effects of cinnamic aldehyde against E. coli O157:H7 and S. aureus PTCC 1337 and its addition had no adverse effects on the sensory attributes of sausages.

The application of phenolic-rich extracts from plants and fruits has also been effectively applied to ready-to-eat meat products such as patties (Ferreira, Morcuende, Hernández-López, et al., 2017; Urrera, Morcuende, Ganhão, & Estévez, 2015), meatballs (Akcán, Estévez, & Serdaroglu, 2017; Wojtasiak-Kalinowska, Onopui, Szpicer, Wierzbiacka, & Półtorak, 2021), and meat stews (Price, Díaz, Banón, & Garrido, 2013), among many others. Ultra-processed muscle foods are subjected to several consecutive procedures such as pre-cooking, cold storage and re-heating. Such harsh conditions promote the creation of an intense pro-oxidative environment that leads to several oxidative damage to both, lipid and proteins (Dominguez et al., 2019; Soladoye et al., 2015). The benefits of using plant antioxidants in some of these ultra-processed meat products include significant reduction of rancidity and off-flavors (Akcán, Estévez, & Serdaroglu, 2017; Akcan, Gökçen, Asensio, Estévez, & Morcuende, 2017; Ferreira et al., 2017), improved texture (Estévez, Ventanas, & Cava, 2005; Ganhão, Morcuende, & Estévez, 2010) and decreased concentration of potentially toxic compounds such as MDA (Selani, Contreras-Castillo, Shirahigure, et al., 2011; Van Hecke, Ho, Goethals, & De Smet, 2017), heterocyclic amines (Persson, Graziani, Ferracane, Fogliano, & Skog, 2003) and cholesterol oxides (de Oliveira, Ferreira, Cople, et al., 2018; Rodríguez-Carpena, Morcuende, Petron, & Estevez, 2012).

The reinforcement of the oxidative stability in meat products enriched with ω-3 fatty acids using phytochemicals seems to be another justified technological strategy owing to the highly susceptibility of such long-chain fatty acids to oxidation. The application of plant phenolics and other antioxidants from plant kingdom have been effectively applied to control lipid oxidation and avoid the onset of fishy flavors in reduced-fat Bologna sausages enriched in α-linoleic acid (ALA) and docosahexaenoic (DHA) acids (Melissa officinalis extract; Berasategi, Navarro-Blasco, Calvo, et al., 2014); in ω-3 enriched salted beef patties (acerola fruit extract, Realini, Guardia, Díaz, García-Regueiro, & Arnau,
3. Use of plant antioxidants in meat products: experimental approach

The research in “natural antioxidants” bloomed during the last two decades, during which a large number of original and review papers were published. Some of them concentrated the attention and citations of many other subsequent articles and therefore, scientific journals have been eagerly publishing papers on this topic of growing interest. In this scenario, meat scientists found a topic in which originality and innovation were apparently easily achieved to build a fruitful and productive career. However, originality and innovation may be irrelevant without rational and applicability. From this perspective, simple “cook-and-look” type of experiments in which a “never-used-before” source of “natural antioxidants” was tested for its ability to reduce TBARS in a given meat product, started to pop up like chanterelles in the early Finnish autumn. While the rational use of a natural source of bioactive compounds is required to provide technological relevance and applicability, a balanced and consistent experimental design is required for scientific rigor. In the following lines, pertinent aspects of the experiments designed to evaluate the efficiency and benefits of added bioactive compounds in meat products are critically discussed (Fig. 1).

3.1. Sources and means of application

3.1.1. Sources of antioxidant phytochemicals

The potential sources of bioactive compounds from plant kingdom are massive. Yet, not all of them may be of interest for the application in particular foods such as meat products. Besides the rational opportunities already addressed in the previous Section 2.2, natural sources of bioactive compounds may fulfill a set of requirements namely, availability, applicability, low price, safety, and effectivity at low concentrations. Further on, the plant source, whether it is a spice, herb, essential oil, crude extract, or phenolic-rich concentrated extract, must be compatible with the selected meat system, in terms of organoleptic properties (i.e. sensory coherence) and technological processes (i.e. effective under the specific processing conditions). In this regard, distinct odours and/or flavors imparted by marjoram, black pepper, rosemary or sage may be compatible with the sensory profile of certain meat products (Embuscado, 2015). Alternatively, deodorized extracts have been applied to avoid consumer rejection while guaranteeing antioxidant effectiveness (Rajeev, Johannah, Gopakumar, Malikel, & Krishnakumar, 2017). Additionally, bioactive compounds must be resistant to high temperatures, hydrostatic pressure, irradiation etc. if the meat products are destined to be subjected to such processes and an antioxidant action is required in further processing/storage. In this scenario, by-products from agricultural industry are appropriate candidates to be used as sources of bioactive compounds (Jimenez-Lopez et al., 2020). Waste materials from the production of juices, wines, beers, jellies, jams and vegetable oils, among others, are produced in

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high quantities and have been reported to contain high quantities of bioactive compounds such as tocopherols, ascorbic acid, carotenoids, phenolic diterpenes and a large variety of phenolic acids and polyphenols (Barbera, 2020; Fierascu et al., 2020). It is worth emphasizing that large quantities of bioactive compounds may also be found in waste materials from animal production, slaughter, and processing (Toldrá, Aristoy, & Mora, 2020a). Yet, the necessity of such manipulation to obtain bioactive peptides from animal-sourced proteins may affect to the paradigms of what consumers identify as “natural”. Whether bioactive compounds are obtained from agricultural by products or animal-sourced waste materials, the advantages of such sources are indisputable in terms of availability, low cost and effectiveness. Further to the aforementioned benefits, using waste materials for their re-utilization in the meat industry deliver additional gains in terms of bioeconomy and environmental protection. Yet, safety concerns of these materials should be specifically addressed for each case as depending on the source, and particularly, the technological processes applied during processing/extraction, particular chemical threats could remain in the ready-to-use bioactive ingredient. Studies aimed to guarantee the safety phytochemicals extracted from plant materials such as the one carried out by Farag, El-Baroty, and Basuny (2003) in olive phenolics should be systematically accomplished to assure the application of safe bioactive ingredients in the meat industry. Caution should be taken with seaweed as source of bioactives given that some nanoplastics may be extracted and introduced into the food chain (Banach, Hoek-van den Hil, & van der Fels-Klerx, 2020).

3.1.2. Application of antioxidant phytochemicals

While the means to apply “natural antioxidants” to meat and meat products are numerous, these strategies can be divided in two major options: dietary means and technological applications. The efficiency of enhancing the oxidative stability of meat animals by supplementing animal feeds with ingredients with antioxidant potential has been known for several decades. Tocopherols and, to a lesser extent, carotenoids, have been proven to accumulate in muscle tissue and protect cell components against oxidative damage (Buckley, Morrissey, & Gray, 1995; Nabi, Arain, Rajput, et al., 2020). The benefits of this strategy in terms of enhanced oxidative stability and improve meat quality is also achieved when animals are fed with tocopherol-rich materials. Again, a number of agricultural by-products are good sources of such bioactive compound such as acorns (Akcan, Gökcü, et al., 2017), chestnuts (Echeagaray, Gómez, Barba, et al., 2018) and olive pomace (Munekata, Nieto, Pateiro, & Lorenzo, 2020), among others. Surplus of certain fruits and vegetables or those rejected for human consumption may also be used for animal nutrition with benefits in terms of health and oxidative status. For illustrative purposes, feeding avocado paste from leftovers to pigs was found to provide lean carcasses and meat with higher oxidative stability upon storage and cooking (Hernández-López, Rodríguez-Carpena, Lemus-Flores, Galindo-García, & Estévez, 2016a; Hernández-López, Rodríguez-Carpena, Lemus-Flores, Grageola-Núñez, & Estévez, 2016b). Antioxidant solutions involving dietary means are interesting in terms of safety as the animal stands as a biological barrier of chemical hazards to humans. Muscle tissue does not accumulate antinutrients and other chemical species of health concern that may be present in plant materials or their extracts. Yet, the physiological processes controlling the growth and biology of muscle tissue is, precisely, a main drawback of the antioxidant solutions involving dietary administration of phenolic-rich plant materials. There is limited evidence of significant accumulations of dietary plant phenolics (or their metabolites) in muscle tissue and hence, a direct antioxidant protection of such bioactive compounds in meat systems is imprecise. While the effect of dietary plant phenolics in muscle tissue may not be comparable to tocopherols and carotenoids, the oral administration of such bioactive compounds may lead to
antioxidant protection in internal organs (including skeletal muscle) by mechanisms that do not involve their direct implication in oxidative reactions. As redox-active species, some polyphenolics have been found to strengthen the endogenous antioxidant defences in experimental and domestic animals (Estevez, Geraert, Liu, et al., 2020; Selby-Pham, Cottrill, Dunshea, et al., 2017). Such physiological effect, derived from the ability of such polyphenols to modulate gene expression through molecular signalling mechanisms, can be reflected in benefits in terms of muscle redox status and meat quality (Jiang & Xiong, 2016; Lee, Lin, Yu, & Lee, 2017). Regrettably, many studies aimed to provide antioxidant protection to meat animals through dietary means ignore these molecular mechanisms which affect the scientific rigor of the study and consistency of the proposed antioxidant solutions. Further discussion on this issue is delivered in the following section.

Further to antioxidant strategies applied at the farm (ante mortem dietary manipulations), meat and meat products can be protected against oxidative reactions using phytochemicals after slaughter, during manipulation, storage and processing of meat and meat products. A large variety of incorporation means have been documented, including direct addition (homogenisation), pulverisation, injection, and incorporation into bioactive packaging and edible films, among others (Estevez & Lorenzo, 2018). The decision making in this regard depend on a number of factors such as the type of meat product (minced, intact, fresh, processed...), type of bioactive ingredient (minced plant material, crude extract, essential oil...) and purpose (protection against discoloration, rancidity, protein oxidation...). While a direct addition of the plant material or crude extract is the most common practise in minced products such as burger patties (Ganhão et al., 2019), meatballs (Suniati & Purnomo, 2019), frankfurters (Vossen et al., 2012), liver pâtés (Estevez, Ventanas, Ramírez, & Cava, 2004), or fermented sausages (Ozaki, Munekata, Jacinto-Valderrama, et al., 2021), the application to intact meats and meat products requires other means such as pulverisation in stakes or cutlets (Lahmar, Morcuende, Andrade, Chekir-Maalmi, & Gomez, 2018; Morcuende et al., 2020) or brine injection in cooked hams (Armenteros, Morcuende, Ventanas, & Estévez, 2016). The incorporation of phytochemicals into edible films elaborated using biopolymers from plant or animal by-products (Ribeiro, Estevinho, & Rocha, 2021) has been found to be not only efficient in a variety of products and processing conditions, but it also further contributes to bioeconomy benefits, as already addressed. Using bioactive packaging materials impregnated with plant bioactives has also been proposed to protect muscle foods against oxidation (Ahmed, Lin, Zou, et al., 2017; Dominguez, Barba, Gómez, et al., 2018). Yet, this experimental approach requires detailed examination of the migration of the bioactive compounds from the packaging material to the meat product. The effectiveness of this sophisticated strategies may be limited by the ability of the phytochemical to migrate, penetrate and hence, occur at significant concentrations on the location where ROS eventually initiate oxidative reactions: in the food matrix (Ahmed et al., 2017; Dominguez et al., 2018). While immobilized antioxidant species may still protect packed meat products by neutralizing ROS occurred in the headspace of the packaging system, further evidence of this mechanism and its effectiveness in meat systems would be appreciated.

3.2. Identification and bioactivity assessment of plant sources

Plant materials with no previous characterisation and limited information on their antioxidant potential, can be assessed for their ability to inhibit oxidative reactions in meat products. Yet, in such improvised experimental design, the molecular basis of the redox reactions is wholly unknown and the occurrence of a positive (antioxidant) effect is left to the sacred geometry of chance. While the outcome could be positive in terms of reduction of oxidation markers in the treated meat product, vs. the control counterpart, the bioactive components and their modes of action would remain indefinite. Ignoring such essential fundamental knowledge hinders taking control of the influential factors and establish consistent antioxidant solutions. Furthermore, a lacking or incomplete characterisation of the plant material affects the safety of the solution as it is ignored the nature, concentration, and biological effects of the phytochemicals and/or the occurrence of other chemical species of toxicological concern. Nowadays, a detailed characterisation is normally demanded by scientific journals for studies aimed to assess the antioxidant potential of plant materials on meat products. This characterisation typically includes the concentration of components of antioxidant potential (tocopherols, carotenoids, ascorbic acid, phenolic compounds...) and in vitro evaluation of antioxidant activity (radical-scavenging, chelating abilities...) (Shahidi & Ambigaipalan, 2015). Regarding the analysis of bioactive components, the quantification of total phenolics (TPC) by the Folin Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999), is, alone, inadequate as a characterisation procedure and needs to be complemented with profiling of phenolic compounds using chromatographic methods. At least, a simple classification of phenolic compounds in the most relevant classes of phenolic acids (hydroxybenzoic and hydroxycinnamic acids) and polyphenols (flavonoids, flavanols, catechins, anthocyanins etc.) (Shahidi & Ambigaipalan, 2015). It is worth emphasizing that TPC does not provide information on bioactivity and ascribing antioxidant potential to the amount of such heterogeneous group of chemical species, is incorrect. Moreover, the methods commonly employed to specifically assess in vitro antioxidant activity of plant materials or their extracts (DPPH, ABTS, CUPRAC), have been recurrently criticized for providing inconsistent information on the actual behaviour of the bioactive compounds in a complex food matrix (Granato, Shahidi, Wrolstad, et al., 2018). Yet, this simple characterisation of the plant material in bioactive components and in vitro bioactivity is essential to perform an in-depth discussion of the results in which the antioxidant mechanisms are identified and properly discussed. Otherwise, inefficient, or unexpected (pro-oxidant) effects could also be explained and subsequently counteracted, by taking the required actions that typically involve modifying extraction method and application dose. Pro-oxidant actions of plant phenolics have been extensively documented in vitro and in meat systems (Eghbaliferiz & Iranshahi, 2016; Zhou & Elias, 2013). These undesirable actions depend on a number of factors including the substrate, the dose, solubility, pH and occurrence of transition metals and other redox-active compounds (Decker, 1997). Only by profiling the bioactive compounds and having a detailed characterisation of the reaction conditions, pro-oxidant actions of certain phenolics may be avoided. Further to that basic characterisation, phenolic-rich plant materials and/or individual phenolic compounds may be subjected to additional analyses in order to gain insight into their antioxidant mechanisms and guarantee safe and consistent antioxidant actions in the selected food item. The solubility of the phytochemicals and hence, their partition coefficients (Kp) into aqueous and lipid phases, largely determines their location and molecular interaction with the substrates in complex food matrices (Rice-Evans, Miller, & Paganga, 1996). The identification of these parameters enables a more precise prediction of the behaviours and effectiveness of particular phytochemicals in meat emulsions or other colloidal systems in which lipid and water phases are clearly defined. The in vitro study of the molecular interactions between phytochemicals and food proteins is, as well, an interesting preliminary approach, particularly when protein oxidation is to be investigated from a mechanistic perspective (Keppler et al., 2020; Xu & Xu, 2021). The redox properties of plant phenolics on proteins is dependent on a number of variables including the oxidation state of the phenolics, its interaction with the substrate and potential covalent linkage of the phytochemicals with meat proteins (Zhang, Cheng, et al., 2020). Such interactions largely determine not only the redox effects on the proteins, but also their functionality (Xu & Xu, 2021), rheological properties (Guo & Xiong, 2021) and nutritional and health effects (Zhang, Cheng, et al., 2020). In this regard, the identification of the redox state of the phytochemical (phenolic form vs. quinone) and the performance of molecular docking studies are highly valuable to understand the chemistry involved in the antioxidant
The experimental setting, analytical methods and discussion of results should be planned to fulfil the specific objectives from each of these 3 different approaches. (A), ii) protect particular meat products against oxidation (B) or iii) deliver benefits to the consumer in terms of improved nutritional value and/or health status (C).

3.3. Methodological approach and inference

The ultimate purpose of scientific studies is the fulfilment of specific objectives that should address issues of scientific and/or technological interest. Obtaining reliable data, performing a reasoned and in-depth scientific discussion of the results and coming to reasonable and well-supported conclusions, require a suitable experimental plan. One main issue that should be considered in any scientific study of experimental nature is the occurrence of true replications. Since this matter is commonly covered in author’s instructions of this and many other Journals, a detailed explanation of their definition and significance may not be required. Yet, it is worth recalling that collecting and analysing observations from repeated experiences is the basis of scientific research and hence, the antioxidant effect of a given plant material on a meat system should be confirmed by replicating the whole experimental procedure several times. Means subjected to statistical analysis should be calculated from measurements made in samples produced from different batches, obtained from independent experiments. While the number of replications and the replicated elements may vary depending on the experimental design and objectives, authors should guarantee the variability in the source and extractability of the plant materials and the treatment to be applied should be carried out in meat/meat products obtained from different animals and or processing batches.

The design of the experimental plan should be fully devoted to the fulfilment of the scientific objectives (Fig. 2). In this regard, a large variety of scientific articles dealing with “natural antioxidants” and “meat systems” are found in literature: i) studies of mechanistic nature aimed to provide insight into the molecular basis of antioxidant actions; ii) studies of straightforward applicability aimed to assess the effectiveness of an antioxidant strategy on a particular meat product and iii) studies of interdisciplinary nature in which the application of the antioxidant solutions aims to provide benefits to the consumer.

3.3.1. Mechanistic studies

The first group of studies are essential to establish the fundamental basis of the antioxidant actions. The knowledge gained in studies of mechanistic nature is of indisputable interest to design the experiments and interpret the results from studies of industrial applicability. These studies are typically devoted to the mode of action of individual phytochemicals, or a selected group of these species and the number of reagents is also kept restricted in a simple in vitro system. The comprehension of complex chemistry requires simplistic model systems in which the scientists may be able to control of potential variables and deliver a detailed description of underlying molecular mechanisms. Therefore, the extraction of meat proteins, sarcoplasmic and/or myofibrillar, in typically preferred to build plain experimental units in which other muscle (i.e. iron, myoglobin, lipids) or non-muscle components (i.e. sodium chloride, nitrite) are combined together with the phytochemical to be tested (Chen, Zhang, Ren, et al., 2020; Lahmar, Akcan, et al., 2018; Lahmar, Morcuende, et al., 2018; Li, Jongberg, Andersen, Davies, & Lund, 2016; Rysman, Utrera, Morcuende, et al., 2016; Rysman, Van Hecke, De Smet, & Van Royen, 2016; Utrera & Estevez, 2013). Oxidation may be induced by using assorted sources of ROS (Utrera &
prior thorough consideration of aforementioned key points, such as the incidence of CVD, cancer and mortality (Zeraatkar, Guyatt, Alonso-Espinosa, 2013). It is worth emphasizing that addition of ingredients, condiments and spices with redox-active components is discouraged in these mechanistic studies. The objective of unveiling precise molecular mechanisms is unrealistic in more realistic and complex meat systems in which the observed effects cannot be ascribed to specific pathways or mechanisms. In order to fulfil this objective, the methodological approach should also be prudently designed, as the depiction of molecular mechanisms requires the analysis of specific chemical species with recognized formation and reactivity pathways. In this regard, the detection and quantification of hexanal, MDA or 4-HNE is preferred to the application of the TBA test (Goethals, Van Hecke, Vossen, et al., 2020). Studies conceived to comprehend the molecular basis of meat discolouration may study myoglobin chemistry instead of analysing instrumental colour (Suman & Joseph, 2013). Likewise, the chromatographic techniques that enable the identification of specific oxidized amino acids such as carbonyls, kynurenines or dityrosines, are more valuable than the routine DPNH method (Hellwig, 2020). In this regard, sophisticated spectrometric approaches, including ‘omics’ techniques (Mitra, Lametsch, Akcan, & Ruiz-Carrascal, 2018) and electron spin resonance (ESR) (Jongberg, Torrgren, Gunvig, Skibsted, & Lund, 2013) have also been successfully applied to provide insight into meat oxidation mechanisms.

3.3.2. Practical application of plant antioxidants: meat quality

The group of articles devoted to describing the antioxidant effects of plant phenolics in meat products is, undoubtedly, the dominant in the scientific literature. The apparent simplicity of testing the antioxidant potential of a ‘never-used-before’ plant material in a given meat product could partly explain the profuse literature on this topic. Yet, conceiving a justified, efficient, and practical antioxidant strategy is not a simple task. In fact, providing data of scientific and technical interest requires a prior thorough consideration of aforementioned key points, such as the justification (identification of the oxidation threat), the selection of an appropriate source of antioxidants, its dose and means of application. Additionally, a pertinent experimental setting should also be designed to guarantee the fulfilment of the main objective in these studies, namely, the actual benefit of applying the antioxidant solution. Such benefit may not only rely on the significant reduction of a particular lipid and/or protein oxidation marker in the treated product vs the control counterpart, if the oxidation level in the latter does not compromise sensory, nutritional or technological properties. Therefore, a credible antioxidant solution may be able to prove the positive effects of the antioxidant treatment on particular quality traits that may be impaired in the control meat product. On this line, it seems unavoidable to support the connection between the oxidative damage and the impaired quality trait by providing scientifically pertinent arguments. Since correlation does not imply causality, finding significant Pearson coefficients or close locations of two parameters in a similarity map is not sufficient evidence of causative nexus. The required convincing claims on the actual benefit of an antioxidant strategy can only be provided from the precise identification of the bioactive components and the understanding of their antioxidant mechanism against the lipid and/or protein oxidative damage. Along with a comprehensive revision of the literature, the selection of the appropriate methods for assessing the extent of the oxidative damage deserves to be carefully considered. The limitations and drawbacks of routine methods such the TBA test for lipid oxidation (Ganhão, Estévez, & Morcuende, 2011; Pérez-Palacios & Estévez, 2019), and the DPNH method for protein oxidation (Estévez, Padilla, Carvalho, et al., 2019; Hellwig, 2020), have been recurrently reported. While the correct implementation of these methods leads to valid results, these need to be discussed considering the inherent restriction of the selected techniques. Complementary methods enable a more comprehensive picture of the oxidation events and the analysis of lipid-derived volatiles has been found to be provide reliable and informative data. Recent studies have reported plausible mechanisms behind the protection of plant phenolics against the discoloration of fresh meat (Honda, Miura, Masuda, & Masuda, 2014) or the onset of rancidity and off-flavours in cooked meat products (Ferreira et al., 2017; Jiang, Zhang, True, Zhou, & Xiong, 2013; Parvin, Zahid, Seo, et al., 2020). On the other hand, protein oxidation is a considerable complex phenomenon, and the application of several complementary measurements is recommended (Estévez, 2011). The depletion of protein components such as thiols and tryptophan, is commonly combined with the accretion of protein oxidation products such as carbonyls and protein cross-links (Hellwig, 2020) recently collected valuable information on the varied methodology to assess protein oxidation in foods and feeds. The application of phenolic-rich plant materials and extracts have been found to effectively counteract the negative impact of protein oxidation on the quality of a variety of processed meat products (Armenteros et al., 2016; Ganhão et al., 2010; Jiang et al., 2013; Ryman, Van Hecke, et al., 2016; Xiang, Cheng, Zhu, & Liu, 2019). It is worth emphasizing that such positive and consistent results are commonly observed when using crude plant materials or their extracts. These crude materials contain a complex mixture of bioactive compounds naturally present in the vegetable source. The nature and relative concentration of phytochemicals in the plant responds to the necessity of fulfilling particular biological functions (i.e. antioxidant/antimicrobial) (Kasote, Katype, Hegde, & Bae, 2015). It is, hence, reasonable that such mixture leads to an overall antioxidant effect when added to a pro-oxidative biological environment such as that created during meat processing/storage. Conversely, using pure phenolic compounds (regardless of their natural or synthetic origin) entails safety concerns and unexpected pro-oxidant actions, particularly when used at high doses (Castaneda-Arriaga et al., 2018). The higher antioxidant effectiveness of phenolic-rich crude extracts over pure phenolic compounds is documented in literature (Jiang et al., 2013; Naveena et al., 2008).

3.3.3. Practical application of plant antioxidants: bioactivity and health

The enrichment of processed meat products in antioxidants from plant kingdom can also be framed in the overall strategies of functional foods design. In this scenario, the meat products may be used as the vehicle to deliver antioxidants that may exert benefits to the consumer. Despite of being generally recognized as sources of essential nutrients and key components of a healthy diet, meat and meat products have received a deceptive campaign of discredit by recurrently associating meat intake with serious health disorders, as reported by Leroy and Key (2013). The negative impact of protein oxidation on the quality of a variety of processed meat products (Armenteros et al., 2016; Ganhão et al., 2010; Jiang et al., 2013; Ryman, Van Hecke, et al., 2016; Xiang, Cheng, Zhu, & Liu, 2019). It is worth emphasizing that such positive and consistent results are commonly observed when using crude plant materials or their extracts. These crude materials contain a complex mixture of bioactive compounds naturally present in the vegetable source. The nature and relative concentration of phytochemicals in the plant responds to the necessity of fulfilling particular biological functions (i.e. antioxidant/antimicrobial) (Kasote, Katype, Hegde, & Bae, 2015). It is, hence, reasonable that such mixture leads to an overall antioxidant effect when added to a pro-oxidative biological environment such as that created during meat processing/storage. Conversely, using pure phenolic compounds (regardless of their natural or synthetic origin) entails safety concerns and unexpected pro-oxidant actions, particularly when used at high doses (Castaneda-Arriaga et al., 2018). The higher antioxidant effectiveness of phenolic-rich crude extracts over pure phenolic compounds is documented in literature (Jiang et al., 2013; Naveena et al., 2008).
concentration of bioactive compounds and therefore, avoid the depletion of the antioxidants during storage/processing of the meat product. Encapsulation has been found to be an effective means to protect dietary antioxidants during meat processing and culinary treatments (Barragán-Martínez, Totosaus, & de Lourdes Pérez-Chabela, 2020). The argument of producing functional foods is reported by many meat scientists to justify the incorporation of phenolic-rich plant materials in meat products. Yet, only few prove the functionality of the developed meat product. As for food labelling, claiming health benefits on a particular meat product from a scientific paper requires scientific evidence of such positive bioactivity. As outlined by Granato, Barba, Bursac Kovacevic, et al. (2020), a novel functional food has to be clinically evaluated to prove the health benefits and guarantee its safety. While testing antioxidant-based functional foods in vitro (cultured cells) and animal models provide supportive data of functionality, conclusive evidence of cause-and-effect in humans require a clinical study. Double-blind randomized controlled clinical trials (RCT) with placebo controls have been identified as the most appropriate tests to address the health benefits and safety of functional foods in human volunteers (Weaver & Miller, 2017). Leading clinical trials, Canales, Benedí, Nus, et al. (2007) reported the benefits of including a walnut-enriched restructured meat product in the diet on the antioxidant status of obese subjects who could, as a result, gain cardiovascular protection. In a subsequent study, the same authors (Sánchez-Muniz, Canales, et al., 2012; Sánchez-Muniz, Olivero-David, et al., 2012) confirmed that the intake of walnut-enriched meat product could protect against CVD by increasing the plasma concentration of antioxidant enzymes, α-tocopherol and decreased the levels of lipoperoxides.

4. Future perspectives

The application of plant antioxidants in meat products will find its future at the frontier of knowledge. The development of indispensable, reliable and safe antioxidant solutions can only be achieved by understanding the molecular basis of the oxidative damage to meat components and the underlying antioxidant mechanisms of phytochemicals. Meat scientists should take steps forward challenges of increasing scientific complexity to guarantee straightforward industrial applications. In the field of redox biochemistry, the connection between lipid and protein oxidation and between the latter and protein nitration requires further clarification. On the same line, the pro-oxidative potential of reducing sugars and Maillard-derived dicarbonyls on meat proteins has to be taken into consideration as glycol-oxidation has been noted as a relevant means of protein deterioration.

From a practical perspective, the future of the application of natural antioxidants in meat products will be strongly influenced by two main factors: sustainability and safety (Fig. 3). Since processing is the main source of oxidative instability and consumers’ trends advice towards the reduction of added additives, the usage plant antioxidants in minimally processed foods, may be feasible means to produce safe “clean-label” meat products. Circular economy will be underlying upcoming decisions and at the farm, exploring the usage of agricultural wastes as feeds is worth from an economic and environmental point of view. From a technological perspective, innovative strategies based on using degradable biopolymers impregnated with phenolics in edible films or bioactive packaging, are antioxidant solutions of future perspective. Encapsulation and the application of nanotechnologies appears to be a field of expansion owing to the interest of controlling the release of bioactive compounds during processing/storage (to protect meat systems against oxidation) or during digestion (meat product used as vehicle of bioactive compounds).

Finally, the joint work of multidisciplinary groups is greatly productive to cover nutritional and health dimensions of meat oxidation and antioxidation. In this regard, very much attention has been given to the fate of meat lipids and proteins during digestion and the impact of meat oxidation on health and particularly aging and chronic diseases. On this line, the use of phytochemicals may fulfill multifaceted objectives in inclusive antioxidant strategies that may not only protect meat against oxidation but actually strengthen gut health and lifespan.

![Fig. 3. The future of the application of natural antioxidants in meat products will be strongly influenced by two main factors: sustainability and safety.](image-url)
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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