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# Lipid oxidation and antioxidant delivery systems in muscle food

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## Abstract

Lipid oxidation accelerates quality deterioration in muscle-based foods (fish, red meat, and poultry), resulting in off-odors/flavors, color problems, texture defects, and safety concerns. Adding antioxidants is one approach to control lipid oxidation, and several delivery strategies have been applied, such as supplementing antioxidants to the feed, direct mixing into minces, or, for whole muscle pieces; spraying, glazing, and injection. However, some issues linked to these technologies hinder their wide utilization, such as low effectiveness, noncompatibility with clean label, and off-flavor. These shortcomings have promoted the development of new antioxidant delivery technologies. In this review, the main focus is on the principles, characteristics, and implementation of five novel antioxidant delivery methods in different types of muscle food products. Their advantages and drawbacks are also summarized, plus comments about future trends in this area. Among novel routes to deliver antioxidants to muscle foods are, for whole tissues, recyclable dipping solutions; for minces, encapsulation; and, for both minces and whole tissues, cross-processing with non-muscle antioxidant-containing raw materials as well as applications of edible films/coatings and active packaging. Advantages of these technologies comprise, for example, low price, the possibility to control the antioxidant release rate, overcoming strong aromas from natural antioxidants, and allowing antioxidant-containing raw materials from the food industry to be valorized, providing an opportunity for more circular food production.

## KEYWORDS

active packaging, cross-processing, encapsulation, edible films and coatings, meat, seafood

## 1 | INTRODUCTION

Lipid oxidation is the main cause of quality deterioration in muscle-based foods (fish, red meat and poultry) after microbial growth (Jiang & Xiong, 2016). In muscle with highly unsaturated lipids and strong pro-oxidative systems, such as certain seafoods, lipid oxidation can even

precede bacterial spoilage (Yu et al., 2020). The oxidation of lipids in muscle results in the development of off-flavors typical of rancidity, ultimately rendering the product unacceptable for human consumption (Ramanathan et al., 2020). Other negative effects are color changes, textural modifications, formation of aldehydes, and a general loss of nutritional quality because of the degradation

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of molecules with both antioxidant and vitamin activity, as well as losses of long chain *n*-3 polyunsaturated fatty acids (LC *n*-3 PUFA). As reviewed by Innes and Calder (2020), consumption of the latter has been positively correlated with the prevention of cardiovascular diseases. Presently, consumer demands for healthy, safe, and convenient muscle-based food products together with demands of the food industry for longer shelf-life have encouraged the development of novel preservation techniques. In the muscle food industry, the use of antioxidants is one of the most common ways to control lipid oxidation. Several conventional antioxidant delivery strategies have been applied for this purpose, such as for minces, direct addition by mixing, or for whole pieces of muscle, spraying, glazing, or injection (Baptista et al., 2020). Also, supplementing antioxidants with diets has been used for farm animals. Although these technologies have many advantages, such as simple processing or minimal equipment investment, further developments in the area of antioxidant additions would increase applicability in the food industry. For example, the direct mixing of antioxidant compounds into minces is limited once the active compounds are consumed in the oxidation reaction, whereupon the protection ceases and the quality of the food degrades at an increased rate (Gómez-Estaca et al., 2014). Further, an excessive amount of antioxidants is added with this technique, given that oxidation is a surface phenomenon, rarely going deeper than 1–4 mm, why there is no chemical motif for adding antioxidants to the center of a food product (Undeland et al., 1998; Undeland et al., 1999). Additionally, direct mixing of antioxidants is indeed limited to minced muscle foods. When it comes to spraying and glazing methods, they may result in antioxidant waste, in the latter case during the thawing (Fadıloğlu & Çoban, 2019). In addition, the unpleasant aroma and odor of some natural antioxidants, such as essential oils and plant extracts, can also be a potential issue calling for masking technologies (Munekata et al., 2020).

Currently, novel antioxidant delivery systems are continuously being developed. For example, the use of recyclable dipping solutions (Wu, Sajib, et al., 2021), cross-processing of muscle-derived products with antioxidant-containing plant or algae raw materials (Abdollahi et al., 2020), antioxidant encapsulation (Bahrami Feridoni & Khademi Shurmasti, 2020) as well as use of edible films, coatings (Shahidi & Hossain, 2020), and active packaging (AP) containing antioxidants (Sun et al., 2021). To the best of our knowledge, there have been no attempts to review, summarize, and compare these more recent antioxidant delivery technologies with the more established ones. Here, we review five techniques from each group, and discuss their principles and their applications into different muscle food products. Finally, potential

advantages and drawbacks of each technology are summarized, and some comments are provided about the future trends.

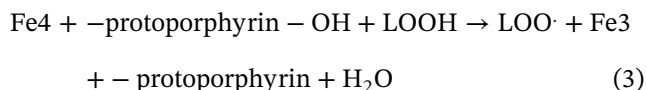
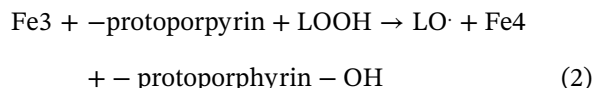
## 2 | MAIN ROUTES FOR LIPID OXIDATION IN MUSCLE FOOD

### 2.1 | Heme protein-mediated lipid oxidation

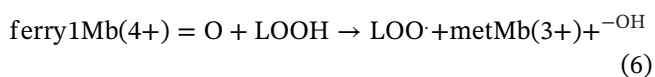
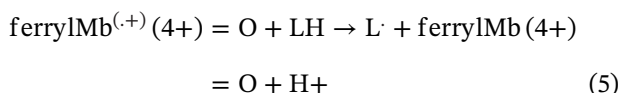
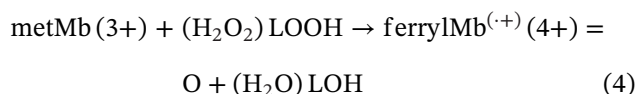
Myoglobin (Mb) of muscle cells and hemoglobin (Hb) from blood in muscle are each capable of promoting lipid oxidation in muscle foods (Chaijan & Panpipat, 2017). Hb and Mb contents in different muscles from poultry, pork, beef, and fish are summarized later, indicating substantial quantities of both heme proteins (HPs) in most of the muscle tissues examined (Table A1). Hb comprises more than 50% of the total HP in muscle from chicken, rainbow trout (*Onchorhynchus mykiss*), Atlantic mackerel (*Scomber scombrus*), chub mackerel (*Scomber japonicus*), and bluefin tuna (*Thunnus thynnus*). Mb comprises more than 50% of the total HP in muscles from beef, pork, turkey, and yellowfin tuna (Table A1). Hb isoforms can be mistaken for Mb causing errantly high estimates of Mb (Grunwald & Richards, 2006a). Mb consists of one polypeptide chain termed globin and one protoporphyrin moiety containing a central iron atom that coordinates ligands such as O<sub>2</sub> (Figure A1). Hb is a tetrameric protein consisting of two alpha chains and two beta chains ( $\alpha_2\beta_2$ ) with each chain having the iron–protoporphyrin moiety reside in a cavity of each globin chain (Mokaberi et al., 2019). The iron–protoporphyrin moiety is also termed heme.

Mb and Hb auto-oxidize to metMb and metHb in which the heme iron changes from the +2 to +3 oxidation state that is associated with the red to brown color change during storage of raw muscle tissue. The brown HPs are collectively termed metHP. It has been repeatedly seen that the reduced forms of Mb and Hb have lower pro-oxidant ability in muscle foods compared to their oxidized form (Wu et al., 2017). There is a fundamental difference between metHb and metMb that involves the bond strength between the globin(s) and the ferri-protoporphyrin IX moiety. Ferri-protoporphyrin IX readily dissociates from metHb at post-mortem pH values (reaction 1), while metMb has relatively high affinity for ferri-protoporphyrin IX. The heme affinity of bovine metHb was 68-fold lower than bovine Mb at pH 5.7 (Cai et al., 2016). Dissociation of ferri-protoporphyrin IX is relevant in the context of lipid oxidation because ferri-protoporphyrin IX released from metHb has been described to intercalate within the phospholipid bilayer of cellular membranes facilitating breakdown of preformed lipid hydroperoxides (LOOH) to alkoxyl and peroxy

radicals (reactions 2 and 3) that propagate lipid oxidation (Shviro et al., 1982; Van der Zee et al., 1996).



The metMb, with a propensity to retain its ferri-protoporphyrin IX moiety, will react with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) or LOOH to form the perferryl Mb radical (reaction 4) (Carlsen et al., 2005). Perferryl Mb radical abstracts a hydrogen atom from a PUFA that propagates lipid oxidation and forms ferryl Mb (reaction 5). FerrylMb can in turn degrade LOOH to  $\text{LOO}\cdot$  and regenerate metMb (reaction 6).



Much attention has been given to protein species that “initiate” lipid oxidation by hydrogen abstraction from PUFA, but it should be kept in mind that preformed LOOH are present in freshly harvested muscle tissue, so that degradation of preformed LOOH to free radical species that facilitate the hydrogen abstraction is likely more critical than hydrogen abstraction by protein species in practical terms of lipid oxidation onset in postmortem muscle tissue.

There are at least two reasons that some fish Hbs promote lipid oxidation more readily than Hbs from terrestrial animals. First, fish metHb (perch and trout IV) were shown to release ferri-protoporphyrin IX up to 54-fold more readily than mammalian metHb at postmortem pH values (Aranda IV et al., 2009). Secondly, the fish Hbs have isoleucine at site E11, which disrupts hydrogen bonding between the distal His and liganded  $\text{O}_2$  that facilitates up to 84-fold faster metHb formation compared to mam-

malian Hbs that contain a smaller valine at site E11 that cannot disrupt the hydrogen bonding (Aranda IV et al., 2009; Brantley et al., 1993). E11 indicates the 11th amino acid residue of the E-helix (Figure A1). As noted earlier, metHb leads to lipid oxidation whereas reduced Hb is considered not oxidative toward lipids until metHb formation occurs. These aspects of fish Hbs may partly explain the greater propensity of fish to undergo lipid oxidation compared to muscle foods from terrestrial animals.

Distinguishing between Mb- and Hb-mediated lipid oxidation in muscle foods is challenging considering that  $\text{H}_2\text{O}_2$  and LOOH can be decomposed by both HPs in promoting lipid oxidation (reactions 2–6). However, the isolated protein apoShp can be used to specifically remove and inactivate ferri-protoporphyrin IX from metHb (Aranda et al., 2007) but not metMb to assess the relative capacity of Hb and Mb to promote lipid oxidation. Added apoShp was shown to inhibit approximately 90% of the lipid oxidation that occurred during iced storage of finely macerated trout whole muscle based on formation of lipid hydroperoxides, LOOH (analyzed as peroxide value, PV), thiobarbituric acid reactive substances (TBARS), and hexanal (Cai et al., 2013); the two latter being the most commonly used measures of secondary oxidation products. This suggested that Hb was the primary oxidant in the finely ground trout muscle. Blood removal by whole body perfusion removed more heme from trout muscle than a bleeding step and more effectively depleted rancid odor formation during storage (Harrysson et al., 2020).

An aspect of pro-oxidative activity that is greater in Mb versus Hb involves auto-oxidation of each HP. Bovine Mb autoxidized to metMb 12-fold faster compared to bovine Hb (Cai et al., 2016). In comparisons of evaluating the lipid oxidation capacity of Mb and Hb from Rainbow trout, Asian carp, and bovine Hb, it was Hb that promoted lipid oxidation in washed muscle model systems more effectively than Mb in all cases (Cai et al., 2016; Richards et al., 2005; Thiansilakul et al., 2012). These findings suggest that the dissociation of the heme moiety from metHb facilitates lipid oxidation (reactions 1–3) more so than lipid oxidation incurred by the relatively rapid auto-oxidation of Mb and the ability of hypervalent Mb to promote lipid oxidation (reactions 4–6). The ability of an Mb variant with low heme affinity to promote lipid oxidation more readily than Mb variants with higher heme affinity further supports the importance of heme dissociation in relation to HP-mediated lipid oxidation (Grunwald & Richards, 2006b).

It should be noted that adding trout Hb to washed muscle facilitated transient formation of ferryl Hb during storage suggesting hypervalent Hb species may also contribute to the totality of lipid oxidation incurred by the fish Hb (Tatiyaborworntham & Richards, 2018). The ability of

$\alpha$ - $\beta$ -unsaturated aldehydes that form during lipid oxidation to accelerate metMb formation should also be considered in the context of Mb as an oxidant (Suman et al., 2006). Further, the roles of mitochondrial metabolites and mitochondrial activity in metMb reduction in postmortem muscle have also been reviewed (Ramanathan et al., 2020). The ability of low oxygen partial pressures to facilitate lipid oxidation should be considered through the ability of deoxyMb or deoxyHb reacting with  $O_2$  to rapidly form metMb and metHb, respectively, and superoxide radical (Ledward, 1970). Superoxide radical can dismutate to  $H_2O_2$  and facilitate lipid oxidation (reaction 4), while metHb and metMb formation incurs lipid oxidation by reactions 1–3 and reactions 4–6, respectively. Notably, trout IV Hb rapidly forms metHb at postmortem pH in part due to low oxygen affinity of the Hb facilitating the reaction of deoxyHb with  $O_2$  (Aranda IV et al., 2009; Binotti et al., 1971).

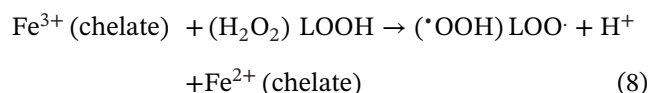
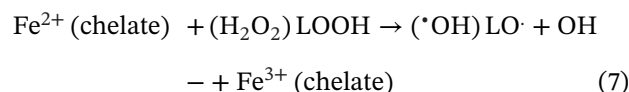
It is commonly believed that lipid oxidation in muscle tissue increases as the unsaturation of the fatty acids within the lipid phase increases. However, there have been cases in which this is not the case. For example, a single source of Hb promoted lipid oxidation in washed tilapia muscle more readily than in washed cod muscle despite the greater unsaturation in the washed cod muscle (Richards et al., 2007). The physical state of the lipid phase may also impact the ability of HPs to promote lipid oxidation. In that, added Mb was unable to promote lipid oxidation in washed pork muscle; however, when the extracted phospholipids were added back to the washed muscle system, the added Mb readily promoted lipid oxidation (Wu, Xiao, et al., 2021). Highly unsaturated phospholipids activated the Mb as an oxidant similarly to phospholipids that were 12-fold less unsaturated (Wu, Xiao, et al., 2021). This again suggested that onset of lipid oxidation in muscle food systems does not always increase with increasing degree of fatty acid unsaturation, and that the physical arrangement of lipids requires further investigation.

## 2.2 | Low molecular weight metals-mediated lipid oxidation

The term low molecular weight (LMW) metal is used since metals are not free in muscle tissue, rather complexed by various molecules that include adenosine diphosphate, sodium citrate, amino acids, and proteins (Chaijan & Panpipat, 2017). Iron and copper are considered the two main metals that facilitate lipid oxidation in muscle food systems. Copper has been described to accelerate oxidation of Mb by a mechanism involving the copper ions binding to histidine residues of the HP to incur metMb formation (Moiseeva & Postnikova, 2001). This demonstrates how

LMW metals can facilitate lipid oxidation by converting HPs to more oxidative forms. Separation of mackerel press juice into the less than 5 kD and greater than 5 kD fractions indicated that both fractions were required for maximal lipid oxidation in a liposome model system (Decker & Hultin, 1990). This suggests that both LMW molecules, including metals, and HMW molecules, including HPs are required to explain the total lipid oxidation capacity of the press juice. The metal chelator  $N',N',N',N'$ -tetrakis-(2-pyridylmethyl)-ethylenediamine (TPEN) was shown to facilitate lipid oxidation in washed cod muscle alone and Hb-mediated lipid oxidation in the washed muscle (Kathirvel & Richards, 2012). This was attributed to the ability of TPEN to activate endogenous metals in the washed muscle which exacerbated lipid oxidation by added Hb. Copper was shown to catalyze the oxidation of Hb at low concentrations while other metals could not even at higher concentrations (Rifkind, 1974).

The reaction of LMW  $Fe^{2+}$  and  $Fe^{3+}$  with LOOH results in formation of alkoxyl radical ( $RO^\bullet$ ) and peroxy radical ( $ROO^\bullet$ ), respectively, while reaction with HOOH results in formation of hydroxyl radical ( $^\bullet OH$ ) and neutral superoxide radical ( $^\bullet OOH$ ), respectively (reactions 7 and 8).



These radicals can facilitate lipid oxidation by abstracting a hydrogen atom from a PUFA. The standard one-electron reduction potential (1-ERP) of  $^\bullet OH$ ,  $RO^\bullet$ ,  $^\bullet OOH$ , and  $ROO^\bullet$  is +2300, +1600, +1060, and +1000  $E^\circ$ , respectively (Buettner, 1993). A more positive 1-ERP indicates a greater propensity to abstract a hydrogen atom from a PUFA. It might seem that  $^\bullet OH$  would be the most conducive to promote lipid oxidation based on its 1-ERP, but  $^\bullet OH$  is so highly reactive that it readily reacts with non-lipid components (e.g., proteins) so that an intermediate 1-ERP may be most oxidative toward lipids.  $Fe^{2+}$  reacts faster with LOOH than  $H_2O_2$  and reaction of  $Fe^{3+}$  with LOOH is slower than with  $Fe^{2+}$  so that adding a reducing agent can increase the lipid oxidation capacity of iron (Halliwell, 2006). Electron-transfer reactions that take place as ascorbate reduces  $Fe^{3+}$  (or  $Cu^{2+}$ ) to  $Fe^{2+}$  (or  $Cu^+$ ) and back to  $Fe^{3+}$  (or  $Cu^{2+}$ ) while generating reactive oxygen species including hydrogen peroxide and neutral superoxide radical has been described (Khan & Martell, 1967).



## 2.3 | Enzyme-mediated lipid oxidation

Enzyme-mediated formations of LOOH in emerald shiner (*Notropis atherinoides*) and rainbow trout (*Salmo gairdneri*) were described to contribute to both fresh fish aromas and those associated with classical autoxidation (Josephson et al., 1987). Lipoxygenase (LOX) activity has been described in muscle tissue as well as skin and gill tissue from fish (German et al., 1986; Jin et al., 2011; Sae-Leaw et al., 2013). LOX in platelets and reticulocytes of blood has also been described (Aleem et al., 2008; Schewe et al., 1981). LOXs generally convert free fatty acids to LOOH which are reactants for LMW metal and HP-mediated lipid oxidation (reactions 2–8). LOX may also act on phospholipids in which fatty acids are esterified to the glycerol backbone (Clark et al., 2011). LOX activity was considered less than that of Hb in a silver carp mince model system with LOX causing fishy odor whereas Hb-mediated lipid oxidation was associated with severe oxidized oil odor (Fu et al., 2009).

## 3 | CONTROL OF LIPID OXIDATION IN MUSCLE BY ANTIOXIDANTS

Antioxidants are compounds that can inhibit oxygen-dependent lipid oxidation, usually by scavenging free radicals, decomposing peroxides, decreasing local oxygen concentrations, or deactivating oxidation initiators, such as Hb, Mb, LOX, or LMW metals (Shah et al., 2014). A grouping of the reaction mechanisms by which antioxidants can deactivate radicals and peroxides revealed they can act by hydrogen atom transfer (HAT), proton coupled electron transfer (PCET), sequential proton loss electron transfer (SPLET), single electron transfer–proton transfer (SET-PT), radical adduct formation, and sequential proton loss HAT (Marković, 2016).

Muscle tissue contains a variety of endogenous antioxidants, both enzymatic (e.g., catalase, superoxide dismutase) and nonenzymatic (e.g. ascorbic acid, tocopherols, carotenoids) components (Dominguez et al., 2019). The level and type of such antioxidants play an important role in preventing oxidative attack of muscle food. For example, our previous study showed that higher contents of ascorbic acid and  $\alpha$ -tocopherol in salmon (*Salmo salar*) backbone compared to in cod (*Gadus morhua*) and herring (*Clupea harengus*) backbones correlated to a high oxidative stability of the former (Wu, Abdollahi, et al., 2021). When supplementing muscle with exogenous antioxidants, it also important to consider the level of endogenous antioxidants, both to allow for potential synergies, but also to not

exceed a threshold when the antioxidants turn into pro-oxidants (Kulawik et al., 2013).

Based on the main pathways of lipid oxidation in muscle food outlined earlier, food scientists or industry can either tailor-make antioxidants and application technologies to specific muscle products or pro-oxidants, or apply more general solutions. Regarding the former, Cai et al. (2013) utilized a recombinant streptococcal protein -apoShp, which has specific affinity for hemin that is released from Hb in fish muscle. The study also investigated the effect of apoShp on lipid oxidation in trout-Hb-fortified washed cod muscle or in minced trout muscle and found that ApoShp could completely prevent Hb-mediated lipid oxidation in the washed cod muscle over 18 days at 2°C storage. In minced trout muscle, addition of apoShp inhibited approximately 90% of the PV, hexanal, and TBARS that developed in controls during 9 days of 2°C storage. These data reinforced that Hb is the primary promoter of lipid oxidation in fish muscle, and points to the need of deactivating it. Thus, antioxidants which can play this role (e.g., caffeic acid, quercetin) should be preferred in fish muscle rather than those being specific to LOX (e.g., esculetin) or low LMW-metals (e.g., chelators as EDTA, citric acid). Although by far the largest number of publications addressing antioxidant additions to muscle foods comprise plant extracts or plant-derived molecules, also animal-derived ingredients as muscle protein hydrolysates (Jonsdottir et al., 2016) or muscle press juices (Cavonius & Undeland, 2017) have been evaluated as natural food antioxidants with great success. It is important to note that some antioxidants could have synergistic effects, which should be taken advantage of when preventing lipid oxidation in muscle food (Nishad et al., 2018). For example, Vieira et al. (2019) reported that a mixture of chitosan and clove essential oil (*Syzygium aromaticum*) (0.16% and 0.08%, respectively) had the highest antioxidant activity and more effectively delayed lipid oxidation in frozen (−18°C) stored tambaqui (*Colossoma macropomum*) fillets compared to either chitosan or clove essential oil alone.

Besides the selection of antioxidants to target the dominating oxidation pathway in the muscle of choice, the delivery method is another core question for the antioxidant's success in different muscle foods. First, this method needs to consider the antioxidant properties, for example, solubility, and stability to heat as well as pH. Also, the form of the targeted muscle product (e.g., minced or intact; fresh or frozen) dictates possible choices and also allows for tailor-made delivery methods to be applied. Conventional choices and more novel methods are discussed in the later sections.

## 4 | CONVENTIONAL ANTIOXIDANT DELIVERY METHODS

### 4.1 | Dietary supplementation for livestock

Antioxidants may be delivered to muscle products via dietary supplementation of farmed animals, which subsequently can improve the oxidative stability and organoleptic properties of the meat (Jiang & Xiong, 2016).  $\alpha$ -Tocopherol is the most traditional antioxidant supplemented via feed, which has been widely reported. For example, Possamai et al. (2018) investigated the effect of supplementing goat feed with vitamin E (50–450 mg/kg dry matter feed) on the stability of its meat toward lipid oxidation during storage at 4°C. The results showed that meat from goats receiving dietary vitamin had a higher level of  $\alpha$ -tocopherol and lower TBARS values. The highest inclusion level provided six days longer storage stability compared to meat from control goats without vitamin E supplementation. Numerous published studies have also confirmed that the addition of plants or their extracts in the diets of fish improves sensory properties and storage stability of the final fish-derived products. Farahi et al. (2012) investigated the effect of feeding rainbow trout with lemon balm (*Melissa officinalis*) and Aloe (*Aloe vera*) on oxidative stability post harvest, and found that both feed additions could protect against lipid oxidation in fish muscle during chilled storage (4°C, 7 days). Although promising with farmed animals, indeed this technique is not applicable in wild-caught fish or other animals.

### 4.2 | Direct mixing into minces

The direct mixing of antioxidants into ground muscle tissues is a common delivery method to control the lipid oxidation of muscle foods (Wu et al., 2020). For example, in our recent study (Wu, Forghani, et al., 2021), seven antioxidative components/formulas were directly mixed into a herring coproduct mince to identify the most promising candidates. Results showed that oil-soluble rosemary extract and iso-ascorbic acid most effectively inhibited lipid oxidation of the herring mince during ice storage. Among the different antioxidants, essential oils (EOs) and plant extracts (PEs) have lately been the most widely studied in muscle food products for controlling lipid oxidation and subsequent rancidity development (Munekata et al., 2020). Baptista et al. (2020) summarized that the concentrations of EO and PE applied in seafood are normally in the range of 0.1–1%, even though higher concentrations (up to 3%) also have been used. The direct mixing of EO and

PE at these levels could generate a potentially unwanted organoleptic effect in the muscle matrix as a strong and unpleasant aroma and odor (Alirezalu et al., 2020), limiting the applicability of this technique. In addition, a relatively long and intense mixing process would be required to distribute the antioxidants in a homogeneous manner, especially if only small amounts are added. It has been proposed that such extended time and intense mixing could affect the physical and chemical properties of the ground mince (Kupchak & Lyubimova, 2017), thereby stimulating oxidation. For example, Sukhenko et al. (2017) investigated the effect of grinding degree on the microstructure of ground meat from beef and pork. The results showed that intensive grinding (12 min) led to a 4–5-fold decrease of muscle fibers' length and the free surface also sharply increased compared to when using normal grinding (1 min). In addition, an amorphous state of the muscle fibers saturated with sufficiently large air bubbles was observed in the ground meat when using intensive grinding.

### 4.3 | Glazing of frozen muscle

Glazing of the muscle food surface is a well-known method generally used to protect products from dehydration and oxidation during freezing and frozen storage (Taheri, 2015). The ice layer, which is applied by dipping or spraying the frozen food item in water, excludes air from the surface of the product and prevents moisture loss, which in turn prevents dried-out surfaces, which can increase the available area for oxidative attack (Solval et al., 2014). Incorporation of natural antioxidants in the form of plant extracts into the glazing liquids has been developed as a widely used antioxidant delivery method for frozen muscle food, which has improved oxidative stability further as compared to water glazing. For example, saponin-free quinoa (*Chenopodium quinoa*) extract was incorporated in the glazing media for Atlantic mackerel (*S. scombrus*) (Trigo et al., 2018), turmeric (*Curcuma longa* L.) extract was used in the glazing media for prochilod (*Prochilodus lineatus*) fillets (Fernandes et al., 2017), and clove essential oil was used in the glazing media for sea bass (*Dicentrarchus labrax*) (He et al., 2019). In our previous study (Cavonius and Undeland, 2017), we investigated the use of herring muscle press juice as a complete antioxidative liquid for the glazing of herring fillets prior to frozen storage. The muscle press juice was produced by high-speed centrifugation of herring mince followed by filtration through a filter paper. Results of our analysis showed that glazing with muscle press juice significantly decreased the formation of PV and TBARS over 52 weeks at –20°C compared to glazing with water

(control). However, overall in this area, few studies have targeted the precise antioxidant mechanism operating when applying glazing as a delivery technology, for example, the migration kinetics of the antioxidants from the ice layer to the frozen muscle, how the antioxidant work in a frozen system, and so on.

#### 4.4 | Spraying of unfrozen muscle with antioxidant solutions

Antioxidants can also be applied onto the surface of non-frozen muscle by a spray mister to ensure uniform coverage (Sveinsdóttir et al., 2020). This technology has been widely used in the meat and fish processing industry as a low-cost and high-efficiency method. As the state of the muscle here is unfrozen instead of frozen, the partitioning of the solution into the muscle will be different, leading to different inhibition results. For example, Pazos et al. (2006) reported that spraying antioxidant solutions (grape procyanidins, hydroxytyrosol, and propyl gallate dissolved in water) onto fresh mackerel fillets before freezing was more efficient than glazing them in a frozen state during subsequent storage at  $-10^{\circ}\text{C}$ . This could be attributed such that spraying antioxidant solutions to the nonfrozen raw material allowed better adsorption, and thus, the phenolic compounds penetrated more deeply into the surface of unfrozen fillets than in the frozen ones, allowing interaction with pro-oxidants and lipids. Similarly, Monirul et al. (2019) investigated the effect of spraying with acetic acid and ascorbic acid on the shelf-life of silver carp (*Hypophthalmichthys molitrix*) fillets at  $4^{\circ}\text{C}$  for 9 days of storage. Results showed that PV and total viable count of microorganism were significantly lowered in fillets sprayed with acetic acid (1.0%) plus ascorbic acid (2.0%) compared to no spraying.

#### 4.5 | Injection of antioxidants

A wide variety of muscle products, including boneless meat, bone-in parts, and fish fillets, are routinely injected with brine for curing to improve tenderness, juiciness, and flavor (Bosse et al., 2021). However, Mariutti and Bragagnolo (2017) have reported that salt added in meat and seafood can favor lipid oxidation. Thus, the delivery of antioxidants along with salt via brine injection has been developed into a common method for marinated meat or fish (Jongberg et al., 2018a). For example, Kozhakhieva et al. (2018) reported that injection of extract from sea buckthorn (*Hippophae rhamnoides*) or seed kernel pumpkin (*Cucurbita pero L.*) at 1.0% (in the finished product) significantly improved the oxidative stability and color character-

istics of horse meat stored for up to 21 days at  $0-4^{\circ}\text{C}$ . Similarly, Jongberg et al. (2018b) investigated the effect of green tea or maté extracts on lipid oxidation in brine-injected retail-packed pork chops. Their results indicated that such injections using 25–160 ppm gallic acid equivalents significantly inhibited the formation of TBARS compared to the control during 7 days of storage at  $5^{\circ}\text{C}$ . However, as stated earlier, the absence of oxidation in the center of muscle pieces implies that injection consumes more antioxidants than needed.

### 5 | RECENT ANTIOXIDANT DELIVERY TECHNOLOGIES

#### 5.1 | Dipping of nonfrozen raw materials in antioxidant solutions

In order to reduce different degradation pathways (i.e., microbial activity, autolysis, lipid oxidation), a water dipping (or rinsing/washing) step is often employed to muscle as a preliminary step prior to chilled storage to remove blood, digestive juices, slime, and/or feces as well as to partially prevent microbial contamination (Miranda et al., 2018). Additionally, dipping solutions have been fortified with antioxidant compounds to enhance such preservative effects (Sveinsdóttir et al., 2020). In this respect, the dipping process provides two opportunities to deliver antioxidants into the muscle food system. Firstly, antioxidants may be adsorbed by proteins or lipids of the muscle-based product, through covalent conjugates, hydrophobic interactions, or electrostatic interactions (McClements & Decker, 2018), a process which to some extent is affected by the immersion time. Secondly, after dipping, the surface of the muscle-food product retains some residual antioxidant-containing solution due to surface tension (Wu, Sajib, et al., 2021), which will continue to exhibit its antioxidant activity during subsequent processing or storage and also provide a certain physical barrier against oxygen. For some antioxidants, such as essential oils which are water insoluble, there is thus a requirement for appropriate food-grade excipients and carriers in the preparation of the antioxidant stock solution. These could be, for example, silicon dioxide, DATEM, propylene glycol, polysorbate 80, monoglycerides of fatty acids, sucro-esters of fatty acids, lecithin, glycerol, gum arabic, modified starch, maltodextrin, vegetable oil, or medium chain triglyceride (MCT) oil (EFSA, 2018). For example, Huang et al. (2018) prepared a dipping antioxidants solution by high shear homogenizing of 0.1% (v/v) essential oil (oregano, thyme, and star anise) and 0.05% polysorbate 80 in order to inhibit lipid oxidation of grass carp (*Ctenopharyngodon idellus*) fillets during subsequent chilled storage ( $4^{\circ}\text{C}$ ). Although the dipping



technology cannot be used for minces, which would disperse into the dipping solution, it works for whole pieces of fish or meat without causing extensive water uptake. For example, we (Wu et al. 2021) recently reported that the moisture content of herring coproducts that underwent dipping in antioxidant solutions was not significantly different to those that did not undergo the dipping treatment.

Since the dipping technology is simple, it has been widely applied to inhibit lipid oxidation and prolong the shelf-life of fish and meat. Karoui and Hassoun (2017) reported that Atlantic mackerel (*S. scombrus*) fillets dipped in a water solution containing rosemary and basil extract (1% of each) resulted in lower levels of volatiles oxidation-derived compounds, PV and TBARS, compared to the control samples during storage at 2°C. Similarly, Kim et al. (2019) reported that dipping of chicken breast in solutions containing 1% lyophilized extracts of scallions, garlic, and gold kiwi resulted in reductions of lipid oxidation rates and microbial growth during storage for 9 days at 4°C compared to dipping in distilled water. In addition, Dekkers et al (2011) used 2% of the <10 kDa fraction of tilapia protein hydrolysates to prepare a dipping solution, and found that dip treatment significantly ( $p < 0.05$ ) reduced the formation of TBARS compared to the control treatment during 90 h of storage at 4°C for mahi mahi fillets. As a way to make this technology more cost-effective, and avoid that the dipping solution is discarded after one treatment, we recently investigated the effects of reusing a dipping solution made of the commercial antioxidant mixture Duralox MANC-213, containing rosemary extract, ascorbic acid, tocopherols, and citric acid, up to 10 times (Figure 1), (Wu, Sajib, et al., 2021). Even after the 10th dip of herring filleting side-streams in this solution, the lipid oxidation lag phase, measured as PV and TBARS, during subsequent storage was extended from <0.5 days to >3.5 days at 20°C, and from <1 day to >11 days at 0°C. From studies in a buffer-based model system, it was seen that Duralox prevented Hb-oxidation and heme-loss, which were likely mechanisms behind the strong effect.

## 5.2 | Cross-processing muscle tissue with antioxidant-containing plant or seaweed raw materials

To date, most coproducts of plants (fruits, berries, vegetables, and seeds) and shellfish (shrimps, crab, etc.) have been utilized as a source of biogas/fuel, livestock feeds, organic fertilizers (Gullón et al., 2020), or, in worse cases are even discarded. However, many of these raw materials are rich in natural antioxidants, such as polyphenols, tannins, flavonoids, flavanols, and carotenoids (Ben-Othman et al., 2020), which is why their use in

production of food ingredients, food additives, or supplements with high nutritional value has gained increasing interest (Kebede & Admassu, 2019). However, many agricultural byproducts are also rich in water, pigments, fibers, flavors, and nutrients, which depending on the intended final food product or process could be limiting, or seen as added values (Panzella et al., 2020). Currently, the most common way to use antioxidants from agricultural byproducts is to recover an extract, which then can be used according to the earlier-mentioned different principles (Lai et al., 2017). Solvent extraction, mechanical procedures, molecular distillation, heating in oil at high temperature, and supercritical fluid extraction are among the methods used for extraction of antioxidants from agricultural byproducts (Brito et al., 2020). However, the production of high purity antioxidant extracts can be costly, hindering their upscaling and full adoption of resulting extracts in the food industry. Another possible approach is to integrate the crude antioxidant-containing byproducts directly into the minced muscle, or during the processing of the muscle (here referred to as “cross-processing”), that is, without a pre-extraction step.

The simplest example of combining plant byproducts with muscle is to mix, for example, juice production press cakes into minced meat/fish products to take full advantage of their antioxidants and the natural synergies between them. For example, mixing cranberry press cake into mechanically separated turkey muscle largely increased the oxidation lag phase from 6 days to 14 days at −4°C (Raghavan & Richards, 2007). Similarly, Damerau et al. (2020) reported that press cakes from Finnish berries were mixed with herring mince which decreased oxidation measured as PV and hexanal to a greater or similar extent than conventional antioxidants (e.g.,  $\alpha$ -tocopherol and L-ascorbic acid) during 10-month frozen storage. A recent review also summarized the direct use of fruit and vegetable “waste,” such as peels and seeds from grape, pomegranate, avocado, and citrus, as antioxidants in meat and its derivatives (Calderón-Oliver & López-Hernández, 2020).

Another approach is to integrate such byproducts during production of muscle-derived products such as surimi, protein isolates, and hydrolysates since the manufacturing of these products involves steps as solution treatment (extraction), homogenization, and separation (Jayatilakan et al., 2012), which could aid efficient delivery of antioxidants into the final products. As a first example of this, Abdollahi et al. (2020) recently reported the production of fish protein isolates using the pH-shift method by cross-processing herring or salmon byproducts with shrimp peeling byproducts and lingonberry press cake. Results revealed that oxidation during the actual pH-shift process was completely prevented in presence of

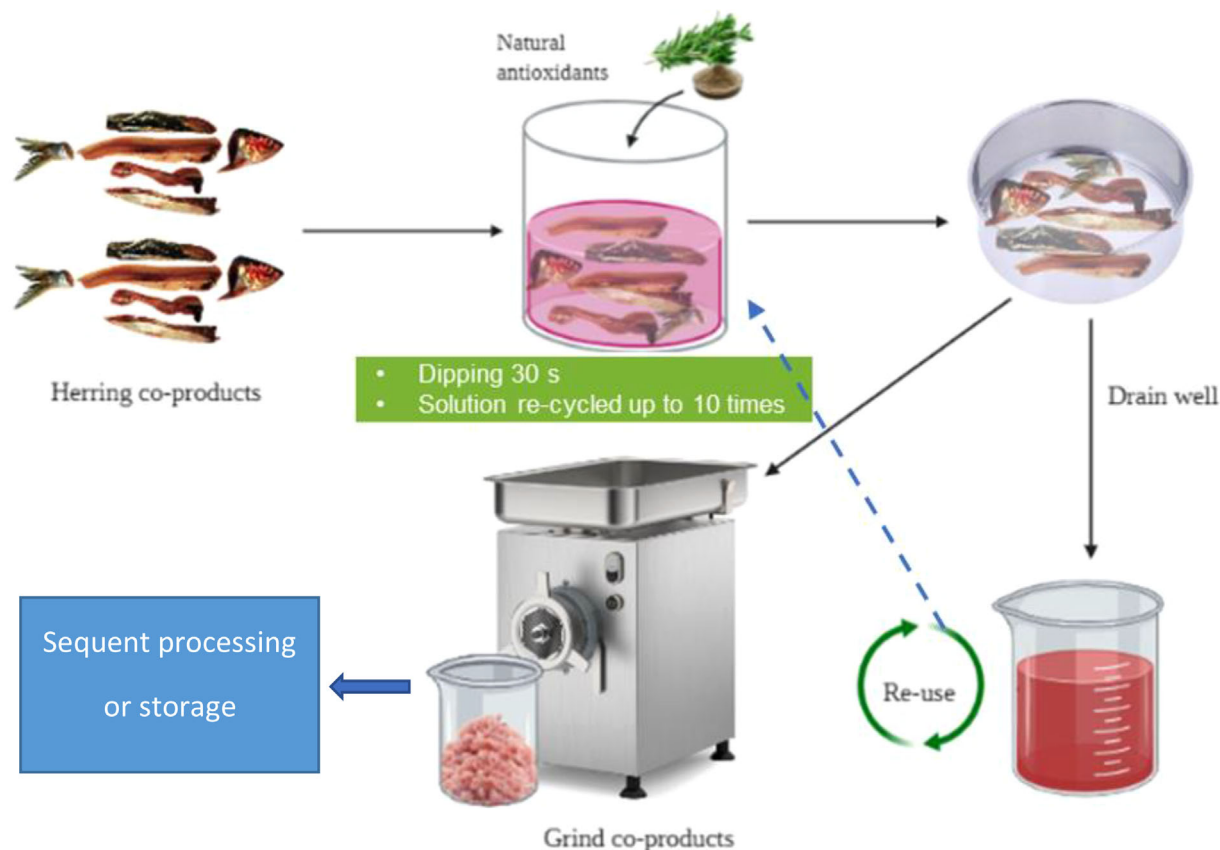


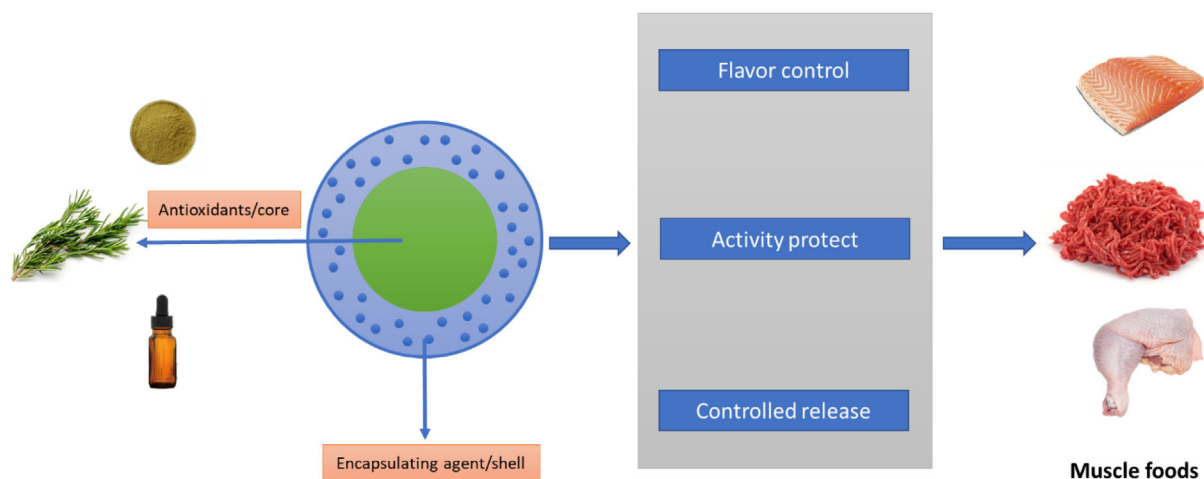
FIGURE 1 Flowchart of the main steps involved in the application of dipping technology for herring coproducts

30% lingonberry press cake (dw/dw) while a small effect also was seen from 30% shrimp peels. Later trials (Zhang et al., submitted) revealed that herring byproduct protein isolates produced with lingonberry press cake were remarkably stable toward lipid oxidation also during subsequent storage on ice; >20 days. Apple pomace from apple juice production also mitigated oxidation during processing and to some extent also during subsequent storage. During pH-shift processing, muscle proteins are solubilized in water under strongly alkaline or acidic conditions followed by isoelectric precipitation (Abdollahi et al., 2021). Given that the tissue is thoroughly homogenized and partly solubilized, water-, acid- or alkali-soluble antioxidants from plant- or algae-materials such as polyphenols can thus partition more easily into oil droplets or interact with proteins/phospholipids via either noncovalent (hydrophobic, ionic, and hydrogen bonding) or covalent bonds (Quan et al., 2019) and form protein-polyphenol conjugates. Conjugates formed by covalent bonds can from some aspects be more preferable in food applications due to their strong and highly stable permanent interactions (Liu et al., 2017). As polyphenols are prone to oxidation under alkaline conditions, they can, in the presence of oxygen, form semiquinone radicals, which subsequently rearrange to form quinones (Schieber, 2018). These reac-

tive intermediates readily react with nucleophilic residues (methionine, lysine, tryptophan, and cysteine) of protein side chains to form covalent crosslinks (C–N or C–S) between the protein and polyphenols enhancing their antioxidant activity (Lund, 2021). Aside from stimulating antioxidant interactions with proteins/phospholipids, cross-processing also efficiently distributes antioxidants into the aqueous phase of the final protein isolates, most often pending between 80 and 90% (w/w) (Wu, Abdollahi, et al., 2021). Although highly promising results are seen from cross-processing fish with, for example, berry byproducts, no quantitative data regarding the actual antioxidant delivery from berry press cake to the fish protein isolate are to date reported, but expected in the near future.

### 5.3 | Application of encapsulated antioxidants

A number of natural antioxidant compounds from aromatic plants are usually highly volatile with low solubility in water and low stability when exposed, for example, to air, light, pH, elevated temperature, and humidity (Gómez et al., 2018). As an example, Arabshahi et al. (2007) reported that the antioxidant activity of extracts from mint



**FIGURE 2** The main goals of microencapsulation: Microencapsulation can be used to achieve material structure, activity protection, and targeted delivery and/or controlled release of the encapsulated antioxidants into the muscle food product

leaves (*Mentha spicata*) and carrot tuber (*Daucus carota*) significantly decreased at pH 4 compared to at pH 9. Further, Ercoli et al. (2021) investigated the antioxidant activity of a colored-flesh potato extract (*Solanum tuberosum*) during storage for 4 months at different temperatures, with or without light. The results showed that at room temperature plus light, the amounts of anthocyanins, that is, the main antioxidant component, decreased by ~85%, while samples stored in the dark at 4°C only lost ~4%. In such cases, encapsulation is a promising technology that enables delivery, protection, and a controlled as well as targeted release of natural antioxidants (Figure 2) into the actual food system (Vinceković et al., 2017). Developments in this area however require fundamental competences in colloid and surface chemistry, material science, and an in-depth understanding of the stabilization properties of the active agents (Alehosseini & Jafari, 2019). The technique can be described as a method of entrapment of a core material (i.e., active ingredient, fill, internal phase, or payload phase) within another solid or liquid immiscible substance, thereby producing capsules with diameters ranging from approximately 10 nm to 10 µm (Froio et al., 2019). Furthermore, the encapsulated systems can be divided into two different forms, namely core-shell type (capsules) and matrix type (spheres) (Đorđević et al., 2015). In the first type, the core material forms a continuous phase enclosed in a shell (liquid or solid), while the matrix type has active compounds uniformly distributed inside a homogeneous solid phase matrix (Gómez et al., 2018). The morphologies of the particles depend on the active and encapsulant materials and the technique used in their preparation.

In the last few years, a number of studies have investigated the encapsulation of natural antioxidants, with the main objectives to preserve their antioxidant properties and promote their targeted action toward oxidative

processes in muscle foods during storage or processing (Gómez et al., 2018). When applying capsules to muscle foods, spraying, dipping, or coating has been frequently used. For example, Mazandrani et al. (2016) recorded less lipid oxidation during storage at 4°C of silver carp fillets dipped in liposomal encapsulated fennel extract (FE) when compared with fillets dipped in solutions of FE in free form or not dipped at all (control). Hadian et al. (2017) reported that nano-encapsulated essential oils from *Rosmarinus officinalis* improved the antioxidant activity in beef cutlet compared to free essential oils. Similarly, Yazgan et al. (2017) reported that encapsulated sunflower essential oil decreased PV and TBARS of both sea bream (*Sparus aurata*) and sea bass (*D. labrax*) fillets during 2°C storage. When investigating the ability of sour tea extract (*Hibiscus sabdariffa* L.) in both free and nanoencapsulated forms to prevent oxidation of chicken nuggets during a 9-day refrigerated storage period (Bahrami Feridoni & Khademi Shurmasti, 2020), the latter was most efficient.

Taken together, these results suggest that encapsulation is a promising technology to deliver antioxidants into muscle foods, thereby improving their quality. Nevertheless, these systems must be carefully formulated so that the antioxidative agents are retained within the capsule and released slowly to maintain their efficacy during extended storage.

## 5.4 | Edible films and coatings containing antioxidants

The increased interest in edible films and coatings has been motivated by increased consumer demand for safe, convenient, and stable foods (Dehghani et al., 2018). An

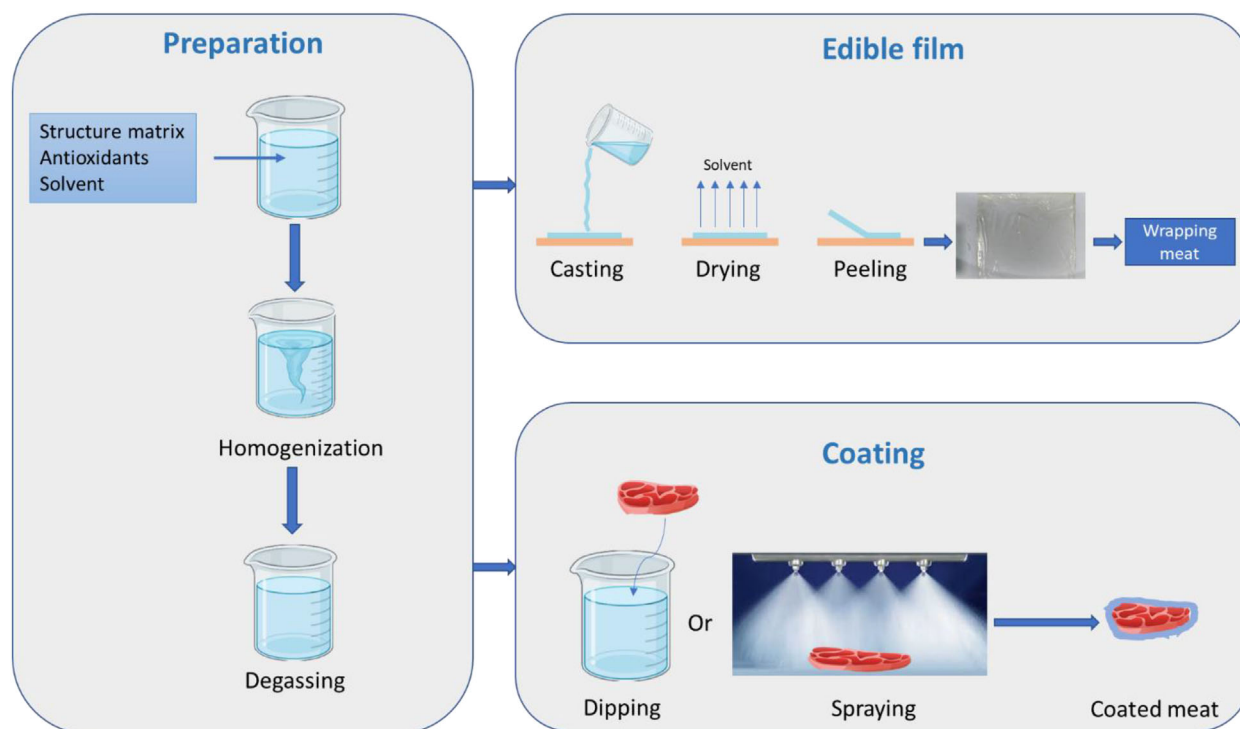


FIGURE 3 Scheme for the preparation of edible films and coatings for muscle food

edible film or coating does not act as a package itself, but provides a layer at the product's surface to prevent moisture loss, release of gas aromas, and solute movement out of the food, while selectively allowing for controlled exchange of important gases, such as oxygen, carbon dioxide, and ethylene, which are involved in food product respiration (Shahidi & Hossain, 2020). Incorporating antioxidants into the film or coating can aid effective and controlled delivery onto the surface of the food item. Further, films and coatings can retard muscle lipid oxidation by absorbing or scavenging undesirable compounds such as free radicals (Dehghani et al., 2018). Notably, although edible coatings and films have a similar definition, there is a difference between them (Figure 3). Generally, an edible coating is a thin layer of edible material formed as a coating on a food product, while an edible film is a preformed, thin layer (i.e., film) made of edible material which, once formed, can be placed on or between food components (Ganiari et al., 2017).

Technologies comprising edible films and coatings enriched with antioxidants have been frequently applied in muscle food preservation research. Normally, the coating includes an impregnation process in which the whole tissue is coated by direct soaking in the solution for a short time (1–5 min) and the excess coating solution is removed from the muscle surface by draining when taking out the muscle from the solution (Umaraw et al., 2020). For example, Choulitoudi et al. (2017) reported that carboxyl methyl

cellulose infused with rosemary essential oil and rosemary extracts was used to prepare coating solutions for smoked eel fillets. The fillets were impregnated in the solutions for 5 min and were then allowed to drain for 1 min. Results showed that both coatings retarded the accumulation of conjugated dienes (CDs), PV and *p*-anisidine value (*p*-AV) during storage at 4°C in a dose-dependent manner compared to coating without antioxidants or no coating. Similarly, Vital et al. (2018) reported that tilapia fillets treated with a coating containing oregano essential oils showed significantly lower TBARS levels and higher sensorial acceptability compared to coating without antioxidants or no coating when stored at  $2 \pm 1^\circ\text{C}$  under light. Also, Chaijan et al. (2020) reported that a whey protein isolate-derived coating including polyphenol-enriched extracts of green tea, lemongrass, and ginger markedly delayed the formation of TBARS, HP degradation, and discoloration of sea bass (*Lates calcarifer*) steak stored at  $4 \pm 0.5^\circ\text{C}$ , thereby extending storage stability from 8 to 15 days. In this area, most studies have focused on investigating the effect of edible films and coatings enriched with antioxidants on lipid oxidation in processed muscle foods. However, very few studies have monitored the antioxidant delivery rate from film to food item, revealing the amount of antioxidant which is actually present during the actual food storage. Gómez-Estaca et al. (2018) reported that a gelatin plus chitosan-derived film containing clove essential oil stabilized salmon (*Salmo salar*) carpaccio against TBARS



during storage at 5°C, and a migration of clove components from the gelatin–chitosan–clove film to the salmon muscle was proven.  $\beta$ -Caryophyllene was identified as the most abundant compound in the film, but eugenol was the compound that migrated most extensively to the salmon. This could be attributed to the highly hydrophobic nature of eugenol, stimulating migration to the fatty salmon muscle.

## 5.5 | Active packaging containing antioxidants

Over the last 10 years, the use of different types of AP materials has been proposed as an alternative to traditional packaging, with two main modes of action: “releasing systems” and “absorbing systems” (Almasi et al., 2020). In the absorbing systems, active compounds of the packaging material absorb compounds from the headspace of the food item which are undesirable from a lipid oxidation perspective, such as oxygen and reactive oxygen species (Wyrwa & Barska, 2017). Since the absorbing systems are not released into the food, the package should be designed to allow access of reactive substances in the food item to the location where scavengers are incorporated (Yildirim et al., 2018).

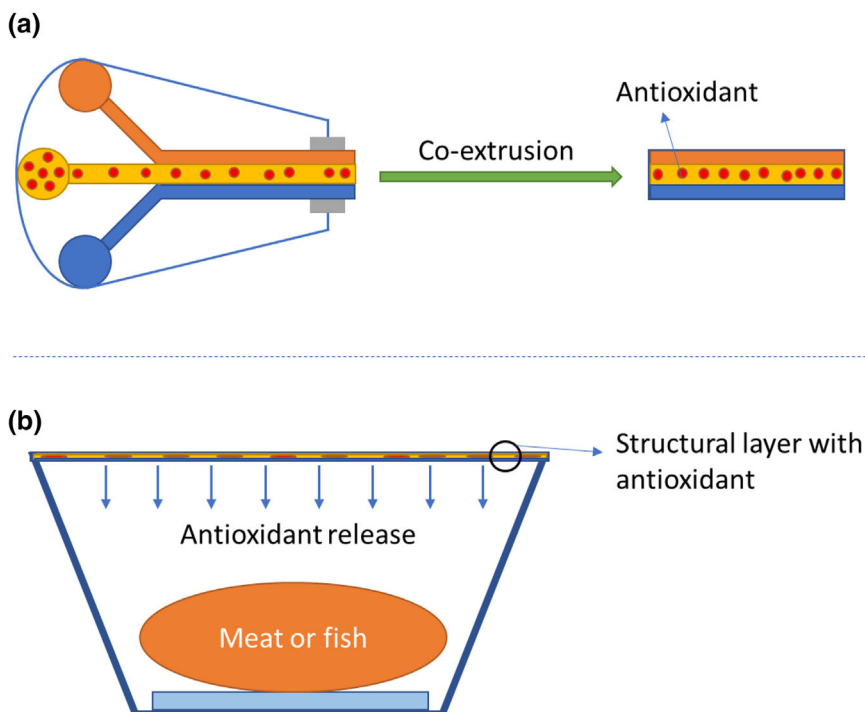
In case of releasing systems, which is the main scope in this review, the selection of antioxidant(s) to be used in the AP material has strict requirements. Although lots of antioxidants have been approved for food use in Europe (Commission, 2011), most of them cannot be used in AP due to three reasons. First, the antioxidants should not decompose in the production of the AP materials, a step which often comprises extrusion. In this process, the antioxidants are added to a melted polymer mass accompanied by temperatures above 100°C, something which indeed decreases the activity of many antioxidants (Domínguez et al., 2018). Réblová (2012) reported that the antioxidant activity of 8 phenolic acids decreased by up to 90% with increasing working temperature (90–150°C). Also the activity of  $\alpha$ - and  $\delta$ -tocopherol decreased with increasing temperature in the range of 110–150°C and both tocopherols were ineffective when subjected to temperatures above 150 °C (Réblová, 2006). Second, the antioxidant and the packaging material should be compatible in order to achieve a homogeneous distribution, and the partition coefficients of the antioxidant in the different phases of the packaging material should favor its release to the food or headspace (Dobrucka & Przekop, 2019). For example, it has been reported that the release rate of quercetin into 95% ethanol was much faster from hydrophilic polymers than from hydrophobic polymers (Chen et al., 2012). Lastly, once released, the effectiveness of the antioxidant

is determined by its solubility characteristics (Shahidi & Ambigaipalan, 2015), and therefore the type of antioxidant should be selected as a function of the type of food. For example, polar antioxidants are more effective in oils, whereas nonpolar antioxidants are more effective in oil-in-water emulsions; often referred to as the “polar paradox” (Noon et al., 2020). Some other factors, such as molecular shape, size, odor, color, cost, and weight of antioxidants, are also important (Dobrucka & Przekop, 2019).

Recently, a wide range of successful studies have been performed on the exploitation of AP systems for meat and meat products (Figure 4). For example, Torres-Arreola et al. (2007) reported delayed lipid oxidation and protein denaturation during frozen storage of sierra fish (*Scomberomorus sierra*) fillets when packed in butylated hydroxytoluene (BHT)-fortified low-density polyethylene (LDPE). Along with the increased demand for natural antioxidants, AP containing such antioxidants has also attracted great interest (Sanches-Silva et al., 2014). Studies on, for example, frozen stored (−20°C) Atlantic salmon (*Salmo salar*) slices, halibut (*Hippoglossus hippoglossus*) fillets, hake (*Merluccius merluccius*) fillets, and blue shark (*Prionace glauca*) slices packed in LDPE films with or without barley husk extract have been carried out (De Abreu et al., 2010, 2011a, 2011b; Pereira de Abreu et al., 2011). Results showed that PV, p-AV, CD, and TBARS were significantly lower in samples packed with barley husk extract-based AP compared to the control samples. Likewise, Dong et al. (2018) reported that AP with LDPE enriched with rosemary and cinnamon essential oils significantly reduced TBARS of shrimp (*Litopenaeus vannamei*) during refrigerated storage. A few studies have explored biodegradable materials produced from biopolymers to replace synthetic polymers, such as LDPE and polystyrene, which are widely used due to their satisfactory physical properties and low cost (Yu et al., 2020). However, most of the biopolymers are highly brittle and permeable to water and vapor, thus limiting their application to foods (Ataei et al., 2020). In addition, the compatibility between biopolymer materials and incorporated antioxidants, and the controlled release of the latter are two challenges that have limited their use.

Controlling the release rate of the active components is a key point for AP and the term “controlled release packaging” first appeared in the literature in 2005 (LaCoste et al., 2005). Among studies addressing the release kinetics of antioxidants from AP, Chen et al. (2012) investigated the feasibility of using polymers with various degrees of hydrophobicity to release tocopherol and quercetin into 95% ethanol (a fatty food simulant). They found that adding tocopherol to quercetin accelerated the release of quercetin from several packaging polymers due to the plasticizing effect of tocopherol. Similarity, Siró et al. (2006)

**FIGURE 4** Schematic representation of methods utilized for the production of multilayer active films (a); graphic representation of active packaging using multilayer or monolayer films (b) The drawing referenced previous illustrations and concepts (Almasi et al., 2020; Domínguez et al., 2018)



incorporated tocopherol/ $\beta$ -cyclodextrin complex into LDPE packaging and showed that the complex slowed down the release of tocopherol due to the low solubility of  $\beta$ -cyclodextrin in the food simulant (95% ethanol). Aytac et al. (2017) conducted a study to incorporate tocopherol into  $\gamma$ -cyclodextrin and then used electrospinning to produce nanofibers from the complex and poly(lactic acid). The release rate of tocopherol was faster for the system with  $\gamma$ -cyclodextrin encapsulation compared to the system without encapsulation since  $\gamma$ -cyclodextrin improved the tocopherol solubility in the aqueous phase of the food simulant. Despite these efforts, more research is needed to obtain release kinetic data for active compounds into real food products, having a higher complexity than food simulants (Chen et al., 2019).

## 6 | ADVANTAGES AND DISADVANTAGES OF THE DIFFERENT TECHNOLOGIES

Table 1 summarizes the reviewed antioxidant delivery technologies' advantages and disadvantages, together with their main characteristics. Simplicity and low-cost are two important factors for industrial choices of antioxidant delivery methods. From Table 1, it is obvious that all conventional delivery methods (e.g., dietary supplementation, direct mixing, glazing, spraying, and injection) include simple-to-relatively simple processes. Also, direct mixing, glazing, and spraying are connected to minimal equip-

ment investments and low cost. These two properties may partly explain why these conventional technologies are still widely used within the muscle foods industry.

On the other hand, with the increasing demands for healthier and safer muscle-based food products, the replacement of synthetic antioxidants (e.g., BHA, BHT, PG, TBHQ) by natural antioxidants (e.g., essential oils and plant extracts) is an increasing trend in the food industry as a whole (Munekata et al., 2020). However, as these natural antioxidants are often unstable under common processing and storage conditions, as well as exhibit strong off-flavors/odors (Gómez et al., 2018), conventional delivery technologies are not compatible. The more recent delivery technologies here show advantages by, for example, stabilizing the antioxidant and masking unpleasant flavors from natural extracts. It has been reported that encapsulation (Vinceković et al., 2017), edible films and coating (Dehghani et al., 2018), as well as AP (Sanches-Silva et al., 2014) all could improve the stability of natural antioxidants and mask their strong flavors and odors. In addition, these three technologies can control the release rate of antioxidants, which could satisfy the demands of the food industry for longer shelf-life. Drawbacks are, however, that these three technologies are costly and require more skills and knowledge, which explain the current gap between the research and commercialization phases.

Besides being compatible with specific antioxidants, the compatibility with various types of muscle food products is another important factor. For example, direct addition via mixing can only be used in minced or dispersed

TABLE 1 Advantages and disadvantages of the different reviewed antioxidant delivery technologies used for muscle food

Delivery method	Main characteristic(s)	Advantages	Disadvantages	References
Dietary supplementation	The natural antioxidants are incorporated into the diet of an animal at a fortified level	<ul style="list-style-type: none"> <li>• Antioxidants distributed to cellular sites where maximum effectiveness may be observed</li> <li>• Initiation of oxidation reactions are limited during harvest and process</li> <li>• Simple process</li> <li>• Widely used for different livestock, not least in aquaculture</li> </ul>	<ul style="list-style-type: none"> <li>• Heat lability of antioxidants requires higher doses in fortification of extruded feeds</li> <li>• High cost as dietary supplementation generally occurs over an extended period, at least several weeks</li> <li>• Limited types of antioxidants can be used, mainly lipophilic and without affecting the flavor profile of the feed negatively</li> <li>• Not applicable to wild animals such as many of our most used food fishes</li> </ul>	Possamai et al. (2018); Cimmino et al. (2018); Cui et al. (2018); Menchetti et al. (2020); Coutinho et al. (2017)
Direct mixing (minced and ground tissues)	Direct addition into minced dispersed material and mixing to reach homogeneous distribution	<ul style="list-style-type: none"> <li>• Minimal equipment investment</li> <li>• Simple one-step process</li> </ul>	<ul style="list-style-type: none"> <li>• A potential issue for off flavor from certain natural antioxidants/extracts</li> <li>• Can only be used in minced/dispersed muscle products</li> <li>• Requires more antioxidant than needed given the surface orientation of lipid oxidation</li> <li>• No assurance of even distribution of the antioxidants</li> <li>• The extra mixing needed can introduce oxygen and by itself stimulate oxidation</li> </ul>	Wu et al. (2021a); Simbine et al. (2021); Özen and Soyer (2018); Wu et al. (2020); Wu et al. (2021b)
Glazing	Antioxidants are included in the ice layer applied to the surface of frozen products by quickly dipping them in an aqueous solution	<ul style="list-style-type: none"> <li>• Simple process</li> <li>• Can be applied to nonuniform intact products</li> <li>• Prevents moisture losses, gas aromas, and solute movements out of the food</li> <li>• Prevents access of oxygen from the air</li> <li>• Targets the surface where lipid oxidation occurs</li> <li>• Low cost</li> </ul>	<ul style="list-style-type: none"> <li>• Only for frozen products</li> <li>• Antioxidant only at surface of product</li> <li>• Some antioxidants will be wasted during thawing</li> <li>• Suitable mainly for water-soluble antioxidants</li> </ul>	Cavonius and Undeland (2017); He et al. (2019); Fadiloğlu and Çoban (2019); Trigo et al. (2018)

(Continues)

TABLE 1 (Continued)

Delivery method	Main characteristic(s)	Advantages	Disadvantages	References
Spraying	The fresh muscle product is subjected to a spray mister to insure uniform coverage of the surface of the product	<ul style="list-style-type: none"> <li>• Simple process</li> <li>• Effective delivery onto the surface of products</li> <li>• Minimal equipment investment</li> <li>• Can be applied to nonuniform intact products</li> <li>• Targets the surface where lipid oxidation occurs</li> </ul>	<ul style="list-style-type: none"> <li>• Uniformity of the sprayed layer can be a problem</li> <li>• A potential issue for off-flavor from natural antioxidants</li> <li>• Some antioxidant solution is lost via spraying “on the side” of the product</li> </ul>	Sveinsdóttir et al. (2020); Monirul et al. (2019); Bonilla et al. (2018); Nguyen (2021)
Injection	The antioxidants are mixed into a curing brine which then is injected into meat or fish	<ul style="list-style-type: none"> <li>• Uniform distribution of antioxidants into the interior of the muscle tissue</li> <li>• Relatively simple process</li> <li>• Can be incorporated into a process line</li> <li>• Recirculation of solution decreases waste</li> </ul>	<ul style="list-style-type: none"> <li>• High-cost equipment and time-consuming</li> <li>• Only applicable to large pieces of muscle</li> <li>• The surface where most oxidation takes place may not be covered</li> <li>• Needles clog if incorporating visual spices or insoluble particles</li> <li>• A potential issue for off-flavor from natural antioxidants</li> </ul>	Jongberg et al. (2018a); Kozhakhlyeva et al. (2018); Choe and Kim (2019); Jongberg et al. (2018b)
Dipping	The muscle is dipped or briefly incubated into an antioxidant solution after which excessive solution is briefly drained	<ul style="list-style-type: none"> <li>• Allow removal of surface bound pro-oxidants as hemoglobin</li> <li>• Wide range of product shapes and sizes can be accommodated</li> <li>• Very simple process and low cost</li> <li>• Delivers antioxidants to the surface where most oxidation takes place</li> <li>• Possibility to recycle the solution for many batches</li> </ul>	<ul style="list-style-type: none"> <li>• A potential issue for off-flavor from natural antioxidants</li> <li>• Some loss of solution while the food item is drained after dipping</li> </ul>	Wu et al. (2021b); Wu et al. (2021c); Miranda et al. (2018); Sveinsdóttir et al. (2020); Huang et al. (2018); Karoui and Hassoun (2017)
Cross-processing	Antioxidant-containing agri-food byproducts or other nonmuscle materials are processed together with muscle raw materials such as during protein isolation or during mincing/mixing of minces products	<ul style="list-style-type: none"> <li>• Low cost if, e.g., byproducts can be used</li> <li>• Promotes sustainable/circular food production by using agri-food byproducts</li> <li>• Allows simultaneous introduction of fibers, nonmuscle vitamins, or phytochemicals, which can improve both texture and nutritional value of the muscle product</li> <li>• May efficiently deliver antioxidants to the active site as the muscle tissue is homogenized/solubilized during processing</li> </ul>	<ul style="list-style-type: none"> <li>• Depending on the actual cross-processing material, potential issues with color, odor, taste</li> <li>• Can interfere with protein yield in the processes</li> <li>• Not applicable if the intended product is intact muscle tissues</li> <li>• Knowledge still limited for this technology</li> </ul>	Abdollahi et al. (2020); Raghavan and Richards (2007); Damerau et al. (2020)

(Continues)



TABLE 1 (Continued)

Delivery method	Main characteristic(s)	Advantages	Disadvantages	References
Encapsulation	Antioxidants are included within another solid or liquid immiscible substance to form a capsule	<ul style="list-style-type: none"> <li>• The antioxidant delivery rate into the food product can be controlled</li> <li>• Prevents off-flavor from natural antioxidants</li> <li>• Antioxidant properties can be protected during food processing</li> </ul>	<ul style="list-style-type: none"> <li>• More skill and knowledge are required to use this complex technology</li> <li>• Limited number of antioxidants suitable for this technology as they should be compatible with capsule</li> <li>• High-cost equipment needed</li> </ul>	Bahrami Feridoni and Khademi Shurmasti (2020); Aytac et al. (2017); Mazandrani et al. (2016)
Edible films and coatings	Antioxidants are included in films or coatings to cover the product	<ul style="list-style-type: none"> <li>• Effective delivery onto the surface of the products</li> <li>• Antioxidant delivery rate into the food product can be controlled</li> <li>• Prevents moisture losses, gas aromas, and solute movements out of the food</li> <li>• Prevents access of oxygen from the air</li> </ul>	<ul style="list-style-type: none"> <li>• Limited number of antioxidants can be used as they should be compatible with film material</li> <li>• Time-consuming on an industrial scale</li> <li>• The dietary allergies and intolerances associated with various protein film-formers</li> <li>• High-cost equipment and film material needed</li> </ul>	Choulitoudi et al. (2017); Vital et al. (2018); Chaijan et al. (2020); Gómez-Estaca et al. (2018)
Active packaging	Antioxidants are included in the packaging material and could be released into the food product in a controlled manner	<ul style="list-style-type: none"> <li>• Prevent unpleasant aroma and odor from natural antioxidants in the actual food product</li> <li>• Control of the antioxidant delivery rate into the food item</li> <li>• No need for extra packaging</li> <li>• Can work according to many different principles; e.g., removing/absorbing reactants or releasing antioxidants</li> </ul>	<ul style="list-style-type: none"> <li>• There is a gap between the active packaging in the research stage and commercialization phase due to high-cost equipment and raw materials</li> <li>• Limited number of antioxidants can be used as the antioxidants should be compatible with packaging material</li> <li>• Knowledge still limited for antioxidant release in the presence of real food materials</li> </ul>	Moudache et al. (2017); Wrona et al. (2021); Kumar et al. (2021); Sun et al. (2021); Li et al. (2019)

muscle products while dipping is only applicable to intact tissues. Coatings and AP on the other hand could be used for both types of muscle. From a sustainable perspective, dipping allows the possibility to recycle the antioxidant solution and cross-processing muscle with, for example, plant food byproducts promotes circular food production.

However, there is still a need to map the economic and technical feasibility of such recent antioxidant delivery technologies by evaluating them also in pilot and full scale.

Taken together, this comparison of different antioxidant delivery technologies hopefully aids their tailoring to different categories of muscle-based products and

makes readers aware of advantages and shortcomings that need to be addressed either in a scientific or industrial level.

## 7 | CONCLUSIONS AND FUTURE TRENDS

Since lipid oxidation in muscle foods is mainly controlled by HPs, the selection of antioxidants should primarily be done to target these potent pro-oxidants, rather than applying broad spectrum compounds or combinations. Regarding modes of delivering antioxidants onto the surface or to the center of muscle, the former appears sufficient because of the surface-oriented nature of lipid oxidation. Although a lot of studies have accumulated regarding the outcome of different antioxidant delivery techniques in terms of reducing lipid oxidation, further studies are still needed to uncover more mechanistic details behind them, as well as the compatibility between different antioxidants and specific delivery technologies. In addition, upscaling lab-scale procedures to pilot and full scale are essential steps to unravel shortcomings which may appear when changing equipment type and batch size. Some of the more recently developed antioxidant delivery technologies put environmental aspects and sustainability in focus, such as the valorization of antioxidant-rich side streams from plant foods processing; others, such as encapsulation technologies, focus on controlling the release rate of antioxidants and preventing the unpleasant aroma and odor of certain natural antioxidants. Here, however, it will always be a trade-off between cost and desired properties, and the value of the end product may dictate which technique can be selected.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

**Haizhou Wu:** Writing original draft; writing review editing; conceptualization. **Mark P. Richards:** Writing original draft; writing review editing; funding acquisition; supervision. **Ingrid Undeland:** Writing review editing; funding acquisition; supervision.

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## SUPPORTING INFORMATION

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## APPENDIX

### Table A1

**TABLE A1** Relative percentages of myoglobin and hemoglobin based on total heme proteins in muscle from chicken, turkey, beef, pork, trout (Rainbow), mackerel (Atlantic and chub), and tuna (yellowfin and bluefin)<sup>a</sup>

	Myoglobin	Hemoglobin	Reference
Chicken (breast) <sup>b</sup>	45	52	(Fleming et al., 1991)
Chicken (breast)	—	100	(Kranen et al., 1999)
Chicken (breast)	—	100	(Rhee & Ziprin, 1987)
Chicken (thigh) <sup>b</sup>	33	60	(Fleming et al., 1991)
Chicken (thigh)	13	83	(Kranen et al., 1999)
Chicken (thigh)	15	85	(Rhee & Ziprin, 1987)
Chicken (mechanically deboned)	23	77	(Lee et al., 1975)
Turkey (breast)	78	22	(Niewiarowicz et al., 1986)
Turkey (thigh)	62	38	(Niewiarowicz et al., 1986)
Beef (L. dorsi)	80	20	(Han et al., 1994)
Beef (L. dorsi)	71	29	(Rhee & Ziprin, 1987)
Beef (Semimembranosus)	67	33	(Rhee & Ziprin, 1987)
Pork (L. dorsi)	69	31	(Pisula, 1975)
Pork (L. dorsi)	53	47	(Rhee & Ziprin, 1987)
Trout (whole) rainbow	—	100	(Richards & Hultin, 2002)
Trout (light) rainbow	0.4	99.6	(O'Brien et al., 1992)
Mackerel (light) Atlantic	—	100	(Richards & Hultin, 2002)
Mackerel (light) chub <sup>c</sup>	—	99	(Matsuura & Hashimoto, 1954)
Mackerel (dark, unbled) Atlantic	35	65	(Richards & Hultin, 2002)
Mackerel (dark, bled) Atlantic	42	58	(Richards & Hultin, 2002)
Mackerel (dark) chub <sup>c</sup>	40	59	(Matsuura & Hashimoto, 1954)
Tuna (light) yellowfin	70	30	(Brown, 1962)
Tuna (dark) yellowfin	88	12	(Brown, 1962)
Tuna (light) bluefin	35	65	(Matsuura & Hashimoto, 1954)
Tuna (dark) bluefin	40	60	(Matsuura & Hashimoto, 1954)
Tuna (deep-seated red) bluefin	38	62	(Matsuura & Hashimoto, 1954)

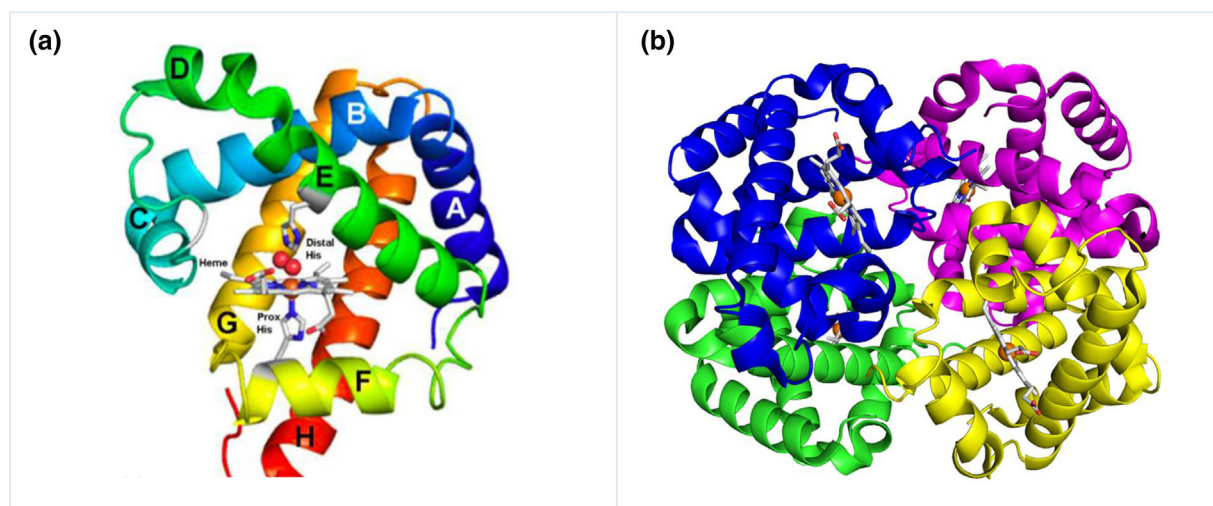
<sup>a</sup>Values shown are the average weight percent of Hb, Mb, or cytochrome relative to the weight of total heme proteins extracted.

<sup>b</sup>Cytochromes in the chicken breast and thigh muscle represented 3% and 6% of the total heme protein content reported, respectively.

<sup>c</sup>Cytochromes represented 1% of total heme protein.

—, Not detected.

Figure A1



**FIGURE A1** (a) Representation of oxymyoglobin (Mb). O<sub>2</sub> (red spheres) is liganded to the iron atom (orange sphere) of the heme ring. The distal histidine (E7) and proximal histidine (F8) are shown in stick representation. E7 indicates the 7th residue on the E-helix and F8 is the 8th residue on the F-helix. (b) Representation of hemoglobin (Hb) an  $\alpha_2\beta_2$  tetramer in which a heme moiety is present in the heme pocket of each globin chain. Chain A ( $\alpha_1$ ), B ( $\beta_1$ ), C ( $\alpha_2$ ), and D ( $\beta_2$ ) are shown in green, blue, yellow, and magenta, respectively. PDB 1MBO (Phillips, 1980) and 1QPW (Lu et al., 2000) were used to prepare the Mb and Hb structures shown, respectively