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CONNECTING POLYPHENOLS AND MYOFIBRILLAR PROTEINS WITH THEIR BIOACTIVE POTENTIALS – A TERSE REVIEW*

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Abstract

The functional properties of animal food protein, especially from both nutritional and health contexts, remain very crucial to (food) product formulation/processing. Moreover, the increases in consumer awareness continues to push the food industry to seek alternatives of chemical food preservatives, particularly those natural approaches able to extend the shelf life of animal (food) products. As such, plant-derived bioactive substances occupy an important space as promising additives for animal food products. Besides, the underlying mechanisms that advance the interactive progress of antioxidant/bioactive compounds within the plant food matrices remain a continued debate. Indeed, to understand how myofibrillar proteins (MP) interact with the specific bioactive ingredients, i.e. biologically active compounds of plant origin, is of great importance, especially their antioxidants/microbial potentials operating as food additives given the eventual impact to modify the functionality/sensory features of the emergent animal food products. To supplement existing information, therefore, this terse review synthesizes some connections between polyphenol and myofibrillar proteins derived from plant-based sources, with emphasis on their anti-microbial potentials. Overall, natural food additives may offer potential health benefits, but their usage comes with challenges, the latter of which animal food producers must carefully consider, particularly the effectiveness of bioactive substances on (animal food) products' safety, and consumer appeal.

Key words: anti-microbial potentials, polyphenols, essential oils, glycosides, myofibrillar proteins

Proteins are macromolecular chemical compounds made of amino acid residues connected by peptide bonds, often divided according to their functions, distinguished by enzymatic, structural, storage, transport, hormonal, receptor, contractile, and defense aspects (Alberts et al., 2002). Proteins remain among key building blocks of living organisms, from the simplest eukaryotes, to more complex prokaryotes (Cozzone, 2002). The spatial properties and structure of proteins further explain the conformation of polypeptide chains. Structurally, protein can be described at four different levels of complexity: primary, secondary, tertiary, and quaternary aspects (Sikorski, 2006). At the primary level, the structure of proteins is depicted by the linkage of amino acid sequence that result to a polypeptide chain. At the secondary level, the structure of proteins is depicted by the location of hydrogen bonds between groups of different peptide bonds, which comprise one elastic chain α helix or two parallel, with separate chains of the same that form a β pleated sheet. At the tertiary level, the structure of proteins is depicted by the polypeptide chain spatially arranged by secondary structure (alpha helices or beta sheets) such that the (protein) macromolecule is stabilized by bonds formed between the side amino acid residues. These include: hydrogen bonds, ionic bonds, disulfide bridges and van der Waals forces, and thus, second- and third-order structures would arise, spontaneously. However, quaternary structure is depicted by the proteins whose molecules comprise from two to several protein macromolecules with a tertiary structure (aggregates) (LaPelusa and Kaushik, 2021; Whitford, 2013; Kyte, 2006).

The functional properties of animal food proteins remain very crucial in (food) product formulation/processing, especially in terms of solubility, emulsifying, foaming, viscosity and gelling aspects (Nielsen, 1997). Protein structure is typically explained by conformation/molecular weight, including such external factors like pH, temperature, ionic strength and the presence of other food ingredients (Liang and Tang, 2013; Demetriades et al., 1997; Southan and MacRitchie, 1999; Schmitt et al., 1998; Lam and Nickerson, 2013). Besides, the functional properties of proteins found within a diversity of raw animal food

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materials, help to shape, not only the desired quality standard, but also the sensory characteristics and technological suitability of emergent meat products, which would directly influence the resultant consistency, emulsifying and gelling properties, water absorption, as well as other biological features (Petracci et al., 2013). Moreover, muscle proteins can be divided into three main group types: myofibrillar, sarcoplasmic and connective tissue proteins. The share of myofibrillar fraction stands at the level of 50–60% of total proteins, which are further distinguished by three subgroups, namely: a) contractile proteins (myosin, actin) – an essential element in the process of muscle contraction and relaxation; b) regulatory proteins (tropomyosin, troponin) – responsible for the initiation and regulation of the contraction phenomenon; and c) cytoskeleton proteins (desmin, nebulin, dystrophin, α-actinin) – constituting the skeleton of the muscle cell, ensuring its integrity and stiffness (Smolińska and Kopeć, 2009).

Researchers continue to search for nutritional/health dimensions of oxidized food ingredients. For instance, the protein oxidation processes in food involve chemical transformations, which include the formation of carbonyl compounds, loss of sulfhydryl groups/tryptophan fluorescence, strengthening of carbonyl derivatives, and formation of intra- and intermolecular cross-linking. The protein oxidation products can accumulate in food during processing/storage, after eating food as found in human internal organs, during subsequent phases of digestion, as well as showing cytotoxic/mutagenic potential (Estévez and Luna, 2017). To prevent negative effects/transformations that facilitate microbial proliferation in animal food products, a number of synthetic preservatives have been used. Examples can include benzoic acid, nitrites and sulfites, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT). Although the (above-mentioned) synthetic preservatives are considered as high preservative with antioxidant that show positive effects on food palatability, some could also show side effects like risk of allergies to benzoic acid and sulfites, which come from chemical reactions when meat products are submitted to heat treatment along with nitrites. In such situations, there could be the formation of carcinogenic nitrosamines, where BHA and BHT may contribute to the risk of cancer (Parke and Lewis, 1992). In addition, the growing awareness of consumers has prompted the food industry to seek alternatives that employ more natural (non-synthetic) approaches for the key purpose to extend the shelf life of animal food products. And this is where bioactive plant-based substances play vital roles, particularly to serve as food additives. Key examples can include glycoside examples like iridoid glycosides, alongside others like terpenes, saponins, polyphenolic compounds (flavonoids, phenols, phenolic acids, phenolaldehydes), tannins, alkaloids, organic acids, mucilages and gums, essential oils, resins and balms, and lignans (Prieto et al., 2022). Evidently, bioactive substances of plant origin (including thyme essential oils, tea polyphenols, rosemary extract, etc.) are shown to possess antibacterial/antioxidant properties (Nwozo et al., 2023; Jagessar, 2019; Daglia, 2012; Silva and Fernandes Júnior, 2010; Rios and Recio, 2005). However, there is still a lot to learn about the underlying mechanisms that underscore the interaction of bioactive compounds within food matrices. Further, a better understanding about how myofibrillar proteins (MP) are able to interact with some (selected) groups of bioactive ingredients is highly warranted, especially when the latter serve as potential antioxidants/antimicrobial food additives, adding their eventual impact to modify emergent products' functionality as well as sensory attributes. To supplement existing information, therefore, this terse review synthesizes some connections between polyphenol and myofibrillar proteins derived from plant-based sources, with emphasis on their anti-microbial potentials.

Protein-polyphenol connection

The highly effective scavenging radicals of polyphenols provide beneficial properties to human health, with the capacity to extend food shelf life, as well as their nature-wide distribution (Adebooyee et al., 2018). Moreover, polyphenols act through several mechanisms beyond their well-known radical scavenging properties. Such mechanisms would include chelating effects, where polyphenols bind metal ions like copper and iron, and are able to prevent catalyzing oxidative reactions. Additionally, polyphenols exhibit reducing effects by donating electrons to neutralize free radicals and, as a result, regenerate other antioxidants, which would further enhance their protective properties. Emphasizing these multiple pathways highlights the diverse and potent antioxidant capabilities of polyphenols, which facilitate their beneficial effects on food preservation and human health (Leopoldini et al., 2011). Polyphenols are organic chemical compounds containing a carbon aromatic ring to which at least two hydroxyl groups are attached. They occur naturally in plants, playing a key role in the growth, regulation and structure of plants, protecting against unfavorable environmental factors, consumption by herbivores or the occurrence of bacterial infections (Zagoskina et al., 2023). Moreover, the use of polyphenols in food production is subject to various laws and regulations, depending on the country and region (Bagchi, 2014). In general, polyphenols are considered safe for human consumption, but there are limits to their use and concentration in food products. In the United States, the Food and Drug Administration (FDA) regulates the use of polyphenols in food production under the Generally Recognized as Safe (GRAS) program. This program allows for the use of certain substances in food production if they have a long history of safe use in food, or if they have been demonstrated to be safe through scientific evidence. Polyphenols that are considered GRAS include those derived from plants such as tea, grapes, and citrus fruits (Abdel-Rahman et al., 2011). In the European Union (EU), the use of polyphenols in food production is regulated by the European Food Safety Authority (EFSA). The EFSA assesses the safety of food additives, including polyphenols, and sets maximum permitted levels for their use in food products (Patanè et al., 2019) (Figure 1).

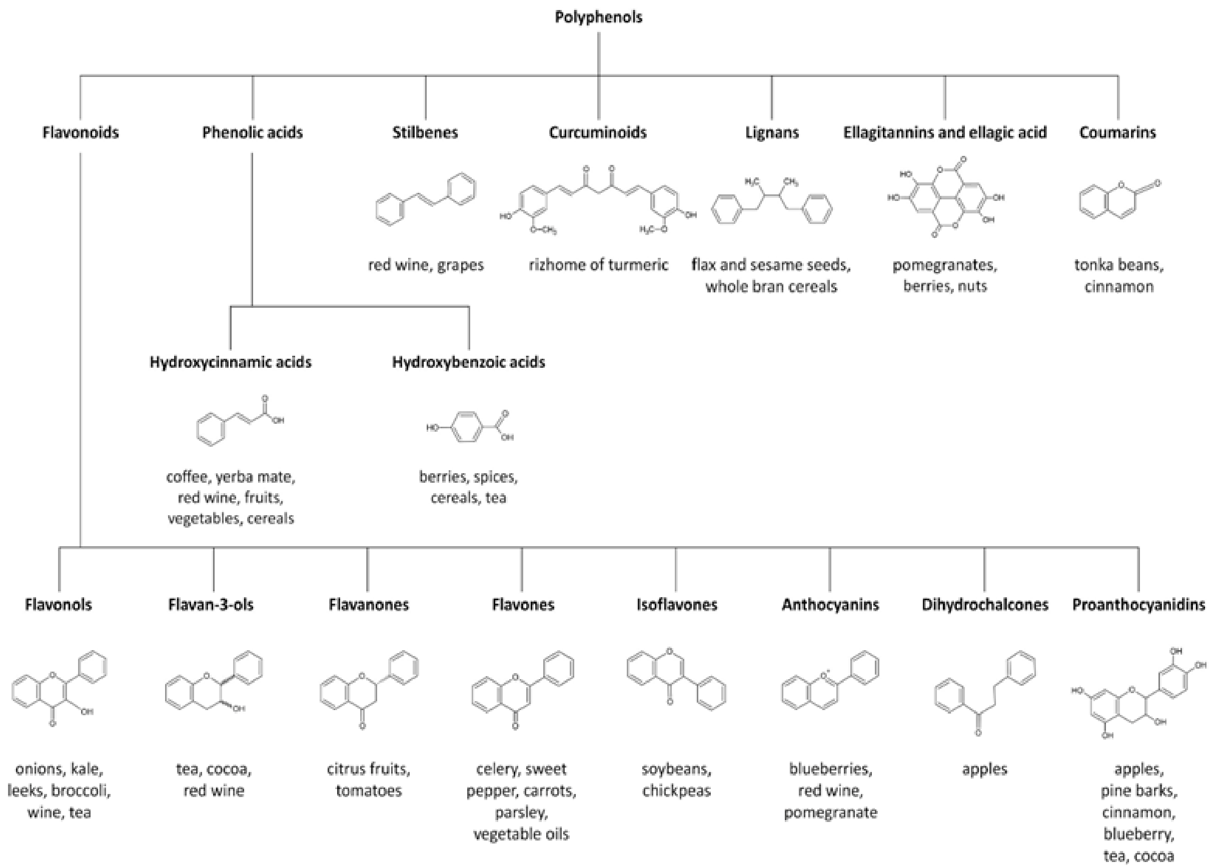

Figure 1. Major polyphenol classes and their most important food sources (Parrella et. al., 2020)

Numerous biochemical, molecular and structural investigations have revealed that polyphenols are able to connect with proteins through the formation of covalent and non-covalent (hydrogen) bonds, which subsequently modify the protein stability/structure, thereby paving the way for physicochemical/functional differences (Cao et al., 2016; Maqsood et al., 2013; Le Bourvellec and Renard, 2012; Jakobek, 2015). The ionized carboxyl groups of polyphenols can form ionic bonds with arginine and lysine residues in proteins. In contrast, oxidized polyphenols can react with amino acid residues such as lysine, tryptophan, methionine, histidine, tyrosine, and cysteine. This reaction occurs through the formation of covalent bonds, as the oxidized phenols interact with free nucleophilic functional groups (like sulfhydryl, amine, amide, indole, or imidazole) as well as the electrophilic groups of quinones (Rawel and Rohn, 2010). Besides, the formation of covalent bonds between proteins and polyphenols would require an irreversible conversion of polyphenols that appear in the form of quinones, which are capable of reacting with nucleophilic groups of protein molecules (Bittner, 2006). Potentially, the presence of van der Waals forces would play a significant role to enhance the progress of phenolic interactions with proteins. Polyphenols, such as kaempferol, quercetin, genistein, and epigallocatechin, have hydrophobic regions that are able to interact with the hydrophobic areas of proteins, like actin. Hydrophobic interactions are an important driving force for these types of interactions, helping to stabilize the binding of polyphenols to proteins (Nagy et al., 2012). Moreover, flavonoids have the ability to change the conformation of actin and form stable complexes (Böhl et al., 2007). For instance, the structure of pork myofibrillar proteins appeared affected by cyanidin 3-O-glucoside, cyanidin 3-O-rutinoside, caffeic acid, quercetin and rutin, as reflected by the remarkable losses of carbonyl and ε-NH_2 groups followed by fluorescence quenching effect (Cheng et al., 2022). Modified myofibrillar protein would increase the antioxidative activity, and enhance the emulsifying properties but decrease the thermal stability. Moreover, caffeic acid would trigger the conversion of α-helix to β-sheet in MP, which is effected through very firm fluorescence quenching, solubility and antioxidant activity. Further, quercetin would show somewhat little effect on secondary structure of myofibrillar protein (Cheng et al., 2020). Besides the inhibitory effect of chlorogenic acid on the formation of protein carbonyl, sulfhydryl and amine groups were lost during oxidation (Cao and Xiong, 2015). However, the presence of polyphenolic compounds that has been intensified oxidatively would be initiated by the loss of α-helix conformation as well as tertiary structure of MP, which by increased surface hydrophobicity and insolubility of proteins would enhance the emulsifying properties (Ozdal et al., 2013).

Notably, the addition of chlorogenic acid up to 30 μmol/g significantly enhanced gelling ability of MP, which formed an elastic gel network by the formation of protein cross-links, reported also for gelatine with various polyphenols (Zhao and Sun, 2017; Maqsood et al., 2013). Oxidized phenolic compounds can be alternative additives that can improve the gelling properties of surimi protein-based or myofibrillar gel products. Balange and Benjakul (2009) demonstrated that adding oxidized tannic acid increased the breaking strength and deformation of mackerel surimi. Additionally, the gel strength of bigeye tuna (*Priacanthus tayenus*) improved at lower moisture content when oxidized polyphenols (0.05% catechin, 0.05% tannic acid, 0.15% caffeic acid, and 0.2% ferulic acid) were used, compared to the control samples that did not contain oxidized polyphenols. Mulberry polyphenols influenced the functional and structural properties of protein through the formation of irreducible covalent bonds and non-covalent bonds with MP. The combination of mulberry polyphenols and MP would promote the protein structure unfolding and aggregation (Huang et al., 2022). Given such interactions, as the carbonyl groups would be formed whereas the total content of sulfhydryl would be reduced, the negative surface charge alongside fluorescence intensity of endogenous tryptophan would be decreased. Further, mulberry polyphenols can increase the degradation of MP gel properties caused by oxidation (Cheng et al., 2020). Other reports have shown the protein-polyphenol conjugates to exhibit improved antioxidant activity over native proteins (Dai et al., 2019). This can be considered to reflect an irreversible interaction of quinone with the sulfhydryl with amino groups of proteins, alongside condensation of quinones, which could lead to the formation of high molecular weight brown pigments to decrease the sensory acceptability of emergent products. Moreover, the reaction of quinone with amino group would decrease the digestibility and bioavailability of amino acids, mostly lysine and cysteine (Quan et al., 2019). Therefore, it is crucial to simultaneously control both the oxidation intensity and dose of polyphenols within meat product processing in order to improve the (meat product) quality.

Myofibrillar proteins – essential oils and glycosides

Myofibrillar proteins comprise certain key components. A strong example is the myosin – very crucial for gelation of meat (proteins), alongside sustenance of three-dimensional structure. Specifically, the myosin molecule comprises two proteolytic fragments, namely: a) heavy meromyosin (HMM); and b) light meromyosin (LMM) (Harrington and Rogers, 1984). Another important component of myofibrillar protein, the actin, helps in strengthening the gel structure of myosin. Further, this actin molecule exists in two forms: G-actin and F-actin (Reisler and Egelman, 2007). Among the myofibrillar proteins, there are both tropomyosin and troponin, which can be distinguished by the no effect on the gelation process. Given the interaction between myosin and actin, the formation of actomyosin complex has a lot to do with the tenderness of meat product (Dara et al., 2021). Thus, the myofibrillar proteins extracted from meat would be a key nutrient source considering the high digestibility (about 92%) and hypoallergenic properties. In fact, their high biological value, which is created by the abundance of both endogenous and exogenous amino acids needed by humans, is accounted for about 70% of the nutritional value of meat (Pereira and Vicente, 2013). Due to their functional properties, the myofibrillar proteins would also be key in helping to realize the emergent meat products (Sun and Holley, 2011). When functional properties of proteins deteriorate, it will be shown by complex oxidation, aging processes as well as microbiological changes (Decker et al., 1993; Xiong et al., 1993; Xiong and Decker, 1995). Further, a negatively-inclined oxidation may affect the nutritional and sensory features associated with meat products (Bao and Ertbjerg, 2015; Villaverde et al., 2014).

Essential oils are liquid, volatile substances with a strong odor, soluble in lipids and organic solvents, with a density lower than water. They are naturally produced by plants to protect them or to attract pollen-carrying insects. They are in the form of a mixture of terpenoids, especially monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20) and other molecules such as acids, alcohols, aldehydes, aliphatic hydrocarbons, aliphatic esters or lactones; less commonly, nitrogen- and sulfur-containing compounds, coumarins and phenylpropanoid homologues. Essential oils are extracted from aromatic plants that are usually found in countries with temperate or warm climates. Due to their antioxidant, antiseptic, healing and fragrance properties, essential oils are often used to preserve food and as analgesics, sedatives, anti-inflammatory, spasmolytic and local anesthetics (Bakkali et al., 2008). Proteins and essential oil components can interact with each other through non-covalent or covalent bonds (Park et al., 2016; Usta et al., 2009). Due to their bactericidal and fungicidal properties, the pharmaceutical and food uses are more and more widespread as alternatives to synthetic chemical products to protect the ecological equilibrium.

The components of essential oils such as oregano, ginger and Chinese pepper (*Zanthoxylum bungeanum*) have a significant protective effect on the oxidation of muscle proteins. This is manifested by preventing the formation of carbonyl compounds and index of fluorescence intensity, which inhibit the activity of endogenous enzymes and reduce the total content of sulfhydryl groups (Lan et al., 2021; Wang et al., 2021). Indeed, polyphenols and essential oils can promote sulfhydryl loss and carbonyl increase through additive reactions (Ali et al., 2018; Mecozzi and Struchio, 2015). Proteins, especially those with NH_2 or NH- groups, are very sensitive to oxidation and are more easily transformed into carbonyls (Sante-Lhoutellier, 2007). Another situation is the carbonyl formation, which is among main consequences of muscle protein oxidation under oxidative conditions (Wang and Xiong, 2008). The use of essential oils of oregano and ginger oil in combination with pectin can effectively slow down the degradation of myofibrillar proteins during cooling, which is able to prevent the occurrence of unfavorable changes in the texture profile by creating new β-sheet structures. This phenomenon occurs due to partial denaturation of the secondary structure of proteins and changes in protein conformation by phenolic compounds between the C-N and N-H groups (Jiang et al., 2022; Zhou et al., 2019). An increase in the concentration of camellia oil from 0 to 40 mg/mL would decrease the α helix, whereas an increase in the β helix, which would lead to the correct unfolding of the secondary structure of the protein, would be beneficial for cross-linking the myofibrillar protein gel by producing a favorable microstructure and increasing the water holding capacity (WHC) (Han et al., 2021). Essential oils as an additive to meat products contribute to reducing losses during cooking, increasing the water retention capacity and delaying MP degradation by inhibiting the reproduction of microorganisms (Lan et al., 2021; Jiang et al., 2022; Calo et al., 2015). They also produce a protective film that hinders the contact of muscles with oxygen, reducing the negative impact of oxidation processes on protein degradation and meat quality (Olatunde and Benjakul, 2018).

According to most reports, meat products with the addition of essential oils are sensory acceptable. The paucity of consumer acceptance may be influenced by individual preferences, concentration and type of essential oils, storage time and temperature (Ruiz-Hernandez et al., 2021). Therefore, the incorporation of clove oil into porcine myofibrillar proteins would delay the deterioration of rheological and structural properties of MP, which are caused by the oxidation of hydroxyl radicals. This would be further manifested by reduced carbonyl formation, loss of Ca-ATPase activity, increased solubility and thermal stability, and, to some extent, improved gel-forming ability (Chen et al., 2016). Elsewhere, white grape extract in beef patties was found to reduce the cross-linking of myosin heavy chains during storage. Through the help of SDS-PAGE analysis, the addition of green tea extract caused a significant loss of myosin and actin heavy chains owed to the formation of thiolquinone (Jongberg et al., 2011).

Glycosides are a group of organic chemical compounds built of sugar and aglycone parts. These are sugar derivatives whose hemiacetal –OH groups at the first carbon atom are replaced by other organic groups. Due to the atom connecting the sugar part with the aglycon, glycosides are divided into C glycosides (connection to the carbon atom), N-glycosides (connection to the nitrogen atom), O-glycosides (connection to the hydroxyl group of the other compound) and S-glycosides (thioglycosides) – the organic group is connected to the monosaccharide through a sulfur atom. Many plant flavonoids are glycosylated (Day et al., 1998; Tohge et al., 2017). Glycosides are linked to the phenolic hydroxyls, via α- or β-D-glycosidic linkages (Murota and Terao, 2003). This type of modification may involve a single oligosaccharide or in some cases a polysaccharide moiety (Xiao et al., 2014). Commonly reported benefits of flavonoid glycosides include anti-oxidants and anti-inflammatory activities, which find useful application in prevention and disease management (Lin and Harnly, 2007; Xiao et al., 2014). The use of glycosides as food additives is regulated by various laws and regulations, depending on the country or region. In the United States, the Food and Drug Administration (FDA) regulates the use of food additives under the Federal Food, Drug, and Cosmetic Act (FD&C Act) and the Code of Federal Regulations (CFR). Glycosides fall under the category of "natural flavoring substances" and are generally recognized as safe (GRAS) by the FDA. However, specific glycosides may require additional safety testing and approval before they can be used as food additives. In the European Union (EU), the use of glycosides and essential oils as food additives is regulated under the Regulation (EC) No 1333/2008 on flavorings and certain food ingredients with flavoring properties for use in and on foods. Glycosides are listed as a subgroup of "flavorings" and are subject to specific regulations regarding their purity, labeling, and usage levels in food products. Glycosides must also be included in the EU's list of authorized food additives before they can be used in food products (Figure 2).

Rutin, also known as rutoside or quercetin-3-O-rutinoside, is a flavonol glycoside compound that has a significant effect on the structure of MP and the properties of MP gel. It was reported that rutin caused the largest cross-linking network and increased the gel strength of pollock skin gelatin gels mainly by interacting with the single-bond CNC backbone and single-bond carboxyl groups of gelatin molecules (Yan et al., 2011). In addition, rutin improves the film strength of soy protein isolate through covalent and non-covalent cross-linking interactions with proteins (Friesen et al., 2015). The side chain group of the MP amino residue was modified, as confirmed by the continuous reduction of the thiol and free amine content and the highest carbonyl content at 200 μmol/g rutin. Routine incorporation resulted in transformations of the α-helix into a β-sheet, a β-turn and a random bundle. The tertiary structure was also changed as observed by a decrease in fluorescence index of internal tryptophan and an increase in surface hydrophobicity. The formation of hydrogen bonds between the hydroxyl groups of rutin and the $–NH_3^+$ group of MP amino acids was strong due to the large molecular size and sugar moieties of rutin and thus favored the increased amount of hydrogen bonding in heat-induced protein gels (Cheng et al., 2020; Friesen et al., 2015). According to Cheng et al. (2020), a distinct spiral pattern was significantly enhanced in the negative band regions of 222 nm after incorporation of rutin, cyanidin 3-O-glucoside, cyanidin 3-O-rutinoside, suggesting the formation of meromyosin light secondary structure. According to Shen et al. (2019), the increase in α-helix content may be due to the binding of phenolic compounds to MP driven by electrostatic and hydrophobic interactions, thus increasing MP stability. Cross-linking of rutin to protein can also be enhanced by the formation of thiol-quinone and amine-quinone adducts, as rutin can be oxidized to form a quinone, which has a higher cross-linking capacity (Jia et al., 2019). Rutin in higher doses is a pro-oxidant (Utrera and Estévez, 2012), which promotes the formation of primary carbonyl compounds, such as α-aminoadipic semialdehyde (AAS) and γ-glutamine semialdehyde (GGS), which may be involved in the formation of oxidative end products. The addition of rutin significantly promoted the formation of oxidized MP carbonyl derivatives and tyrosine dimer, but did not prevent the loss of sulfhydryl groups (-SH). A moderate degree of protein oxidation, especially in the form of protein carbonylation, probably has a beneficial effect on the gelation of myofibrillar proteins (Estévez, 2011). The appropriate dose of rutin (10–100 μmol/g protein) can improve the gel properties of MP due to moderate rutin-induced MP-MP interactions and cross-linking capacity of rutin, with a slight decrease in WHC.

Hesperidin is a bioflavonoid glycoside and is a sugar-bound form of the flavonoid hesperidin and the glycoside rutin whose antioxidant capacity is due to its large number of hydroxyl groups. The addition of orange fiber to dried pork sausages has a protective effect against oxidation, and by reducing the residual level of nitrites, it can prevent the formation of nitrosamines and nitrosamides (Fernández-López et al., 2007). According to Martínez et al. (2019), the chemical structure of hesperidin can explain the radical scavenging activity of citrus also in model studies of pork model system oxidized by a hydrophilic or lipophilic radical initiator, protecting the thiols from oxidation and keeping the thiol concentration comparable to that of non-oxidized control. Gravador et al. (2014) also showed promising antioxidant effects of dried citrus pulp, rich in naringin and hesperidin, when incorporated endogenously into the lamb diet (Barbieri et al., 2019).

Quercitrin – another form of glycoside, which capably induces lower β-sheet content in the myofibrillar proteins, would strengthen the hydrogen bonds via electrostatic and hydrophobic interactions. Quercitrin impairs the solubility of MP, mainly due to an increase in the hydrophobicity of the protein surface (Cheng et al., 2020), which would influence the gel properties of MP, the latter defined by less active center, helical structure, and higher content of quercitrin. However, the significant increase in emulsification stability that occurs further reflects the less developed secondary structures and higher surface hydrophobicity. The presence of sulfhydryl groups in MP modified with quercitrin testifies to their antioxidant properties. Xu et al. (2021) further understood that phenolic compounds, like quercitrin and quercetin, have great potential as natural additives and useful in modifying the functional properties of minced meat products. Accordingly, while the emulsifying properties significantly decreased, the solubility, gel properties, and oxidation stability of MP would improve. Moreover, glycosides commonly used as natural sweeteners, such as imogroside and stevioside, may also have antioxidant properties, although rarely used in food processing especially in the preparation of meat products. He et al. (2022) revealed that stewed pork with the addition of imogroside and stevia glycoside has, above all, some color-protecting and antioxidant properties. However, there is paucity of information confirming the effectiveness of the above-mentioned compounds especially in interaction contexts of myofibrillar proteins (Table 1).

Some anti-microbial potentials of polyphenols, essential oils and glycosides

Scientific literature about the antimicrobial potential of polyphenols, essential oils and glycosides especially against various microorganisms is on the rise, especially those involving bacteria, fungi, and viruses. Further, the use of natural substances continues to increase as an alternative to synthetic antimicrobial agents, which would often bring about bacterial resistance (Chandra et al., 2017). In recent years, there has been greater emphasis on natural antimicrobial compounds, given their potential applications in medicine, agriculture/food industry, as well as cosmetics, although their effectiveness may vary depending on the concentration, method of application, and microbial type (Bolouri et al., 2022). The action mechanism of antimicrobial substances, ranges from damaging cell walls, disrupting the function of cell membranes, inhibiting enzymes and proteins, to interfering with genetic material (Bensid et al., 2022) (Figure 3).

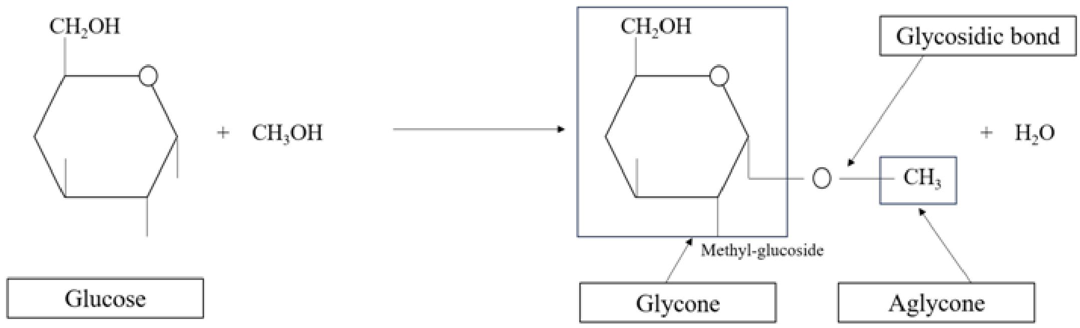

Figure 2. Structure of glycosides

Table 1. Summary of chemical compounds in polyphenols, glycosides, and essential oils and their corresponding effects on myofibrillar proteins

Group of compounds	Chemical compound	Effects on myofibrillar proteins	References
Polyphenols	Catechins	Increasing protein stability, inhibiting lipid and protein oxidation	Xiong et al., 2000; Tang et al., 2001
	Quercetin	Increasing protein solubility, improving meat texture	Roura et al., 2006; Huang et al., 2011
	Epicatechin	Protection against protein denaturation during storage, inhibition of oxidation reactions	Decker et al., 2002; McCarthy et al., 2004
	Gallic acid	Reduction of protein oxidation, protection against enzymatic degradation	Kim et al., 2003; Jiang et al., 2014
	Apigenin	Binding to myofibrillar proteins, improving protein structure and maintaining biological functions	Zhang et al., 2012
Glycosides	Salicin	Stabilization of protein structure, antioxidant effect	Xiong et al., 2011; Liu et al., 2015
	Digitoxin	Increased thermal stability of proteins, protective effect against denaturation	John et al., 2008; Kumar et al., 2012
	Rutoside	Binding to proteins, improving solubility and texture	Rani et al., 2013; Singh and Jain, 2017
	Stevioside	Protection against protein oxidation, preservation of biological functions	Mendoza et al., 2014; Garcia et al., 2018
	Aesculin	Reduction of protein oxidation, anti-inflammatory effect	Jones and Zhang, 2010; Zhang and Wang, 2016
	Hesperidin	Inhibition of protein denaturation, protection against oxidative stress	Li and Zhang, 2011; Zhao and Li, 2019
Essential oils	Rosemary oil (*Rosmarinus officinalis*)	Increased protein stability, antioxidant effect, reduction of lipid oxidation	Sebranek et al., 2005; Nieto et al., 2010
	Thyme oil (*Thymus vulgaris*)	Protection against protein denaturation, improvement of protein texture and stability	Pintado et al., 2010; Baranauskiene et al., 2013
	Oregano oil (*Origanum vulgare*)	Inhibition of protein oxidation	Rojas and Brewer, 2006
	Mint oil (*Mentha piperita*)	Stabilization of myofibrillar proteins, protection against oxidation	Biswas et al., 2012; Hussain et al., 2013
	Lavender oil (*Lavandula angustifolia*)	Increasing the thermal stability of proteins	Cava et al., 2007
	Tea tree oil (*Melaleuca alternifolia*)	Protection against oxidative stress and protein denaturation	Kim et al., 2011

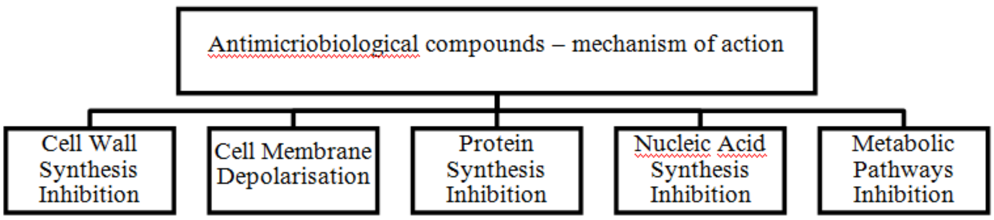

Figure 3. Mechanism of action of antimicrobiological compounds

The antimicrobial activity of polyphenols is underpinned by some key factors, from their potential of disrupting microbial cell membranes, up to inhibiting microbial enzymes (Cushnie and Lamb, 2005). Polyphenols could depict antioxidant/anti-inflammatory properties, which may contribute to their antimicrobial effects. Certain polyphenols, such as epigallocatechin gallate (EGCG) found in green tea, had potent antimicrobial activity against several bacterial species, including *Escherichia coli* and *Salmonella typhimurium*. Besides, EGCG would prevent the proliferation of various Gram-positive and Gram-negative bacteria that lead to food spoilage, despite the antioxidant effects exhibited in food systems (Nikoo et al., 2018). Further, polyphenols can bind to bacterial cell membrane lipids, causing destabilization and increased permeability. Catechins, present in green tea, can depolarize the bacterial cell membrane, leading to leakage of contents and bacterial cell death (Sirk et al., 2009).

Phenolic-rich extracts from fruit are able to inhibit the growth of pathogenic bacteria besides being effective antibiotic resistance modifying agents in pathogenic bacteria (Lima et. al., 2019). Although antimicrobial effect on common foodborne pathogens for glycosides has been studied on a small scale, there are still a number of published works that support the antimicrobial effect of the tested glycosides. By investigating the effect of adding hesperidin on microbiological quality during 171 days of cold storage, Feng and Arai (2022) found sausages (with hesperidin) significantly inhibited the increase of total viable microorganisms. Liao et al. (2022) showed aucubin concentration of 40 mg/mL extract with strong antibacterial activity against *S. aureus*, *E. coli*, *B. subtilis* and *Salmonella.* Overall, natural bioactive compounds, particularly isolated from such plants, promised significant antimicrobial potential against a wide range of microorganisms. However, a better understanding about the mechanisms of action of polyphenols, essential oils and glycosides would help provide the best outcomes as natural antimicrobial agents in food, pharmaceutical and other applications. A number of polyphenols, including quercetin and epigallocatechin gallate, were shown to effectively inhibit the replication of the influenza virus *in vitro*. Besides, the therapeutic effect of polyphenols in different models of influenza virus infection suggests polyphenol-rich plants as a novel natural source for developing future anti-influenza drugs (Bahramsoltani et al., 2016). Natural phenolic-rich extracts, not only would extend the shelf-life of foods by controlling bacterial contamination, but also, coexist with probiotic lactic acid bacteria in food systems to provide humans with enhanced health benefits (Oulahal and Degraeve, 2022).

Mechanism types of antimicrobial action in relation to bioactive compounds

The mechanism types of antimicrobial action and their respective examples specific to polyphenols, essential oils, and glycosides are summarized in Table 2. The mechanism types shown include: a) inhibition of cell wall synthesis (Higuchi et al., 2024; Li et al., 2016; Carson et al., 2006; Sharifi-Rad et al., 2018; Podolak et al., 2010; Zorić and Kosalec, 2022), b) depolarization of cell membrane (Sirk et al., 2009; Alfredsson et al., 2014; McKay and Blumberg, 2006; Marchese et al., 2016; Hostettmann and Marston, 1995; Podolak et al., 2010), c) inhibition of protein synthesis (Baur and Sinclair, 2006; Li et al., 2016; McKay and Blumberg, 2006; Carson et al., 2006; Chatsudthipong and Muanprasat, 2009; Zhang et al., 2012), d) nucleic acid synthesis inhibition (Alfredsson et al., 2014; Sato et al., 2011; Sadlon and Lamson, 2010; Carson et al., 2006; Chatsudthipong and Muanprasat, 2009; Zhang et al., 2012) and e) metabolic pathways inhibition (Baur and Sinclair, 2006; Li et al., 2016; Marchese et al., 2016; Cavanagh and Wilkinson, 2002; Podolak et al., 2010; Zhang et al., 2012).Whilst some polyphenols like quercetin would be involved in inhibition of cell wall/protein synthesis, and metabolic pathways, the glycosides like saponin would be involved in only the inhibition of cell wall synthesis and metabolic pathways (Li et al., 2016; Podolak et al. 2010). Besides, the mechanism types of antimicrobial action specific to essential oils would relate to how it is able to penetrate through the bacterial cell wall, as well as how it would attack the phospholipid bilayer within (bacterial) cell membranes. When such situation occurs, there is a high chance for increased permeability and leakage of the cytoplasm to occur, which allows for some form of enzymatic interaction within the bacterial cell wall (Emiroğlu et al., 2010). In addition, the components of the essential oils could influence the genetic constituents/materials in the microbes, which are relevant in both fatty acid production and electron transport. For instance, clove oil – an additive to casings, produced from myofibrillar proteins and isolated from poultry waste materials, showed higher antibacterial activity against *B. subtilis, S. aureus* and *E. coli*, and thyme oil against *L. monocytogenes* (Saricaoglu and Turhan, 2020). On the other hand, the basil essential oil as an addition to the protein casings as isolated from fish muscles would be characterized by enhanced inhibition of proliferated Gram-positive bacteria (*L. monocytogenes*) when compared to Gram-negative bacteria (*P. aeruginosa*) (Arfat et al., 2014). Besides, it would be promising phenolic extracts isolated from calyxes of *Hibiscus sabdariffa* L. would show some antimicrobial effect on food-borne pathogenic bacteria like those that revealed increased shelf life of animal food products, such as beef (Márquez-Rodríguez et al., 2020). There is the need for essential oils to attain sufficient antimicrobial activity especially in animal products that are rich in fat and protein. As such, there would be the requirement of higher concentrations compared to *in vitro*, which can significantly reduce the sensory appeal of the animal food products (Huq et al., 2015; Yostawonkul et al., 2021) (Table 2).

Across essential oils, there are some key components that would possess antimicrobial properties. Major examples include: a) aldehydes (such as cinnamaldehyde – oil from cinnamon bark/leaves, oil from cassia bark); b) ketones (such as menthone – oil from mint leaves), esters (such as linalyl acetate – essential oils from lavender, jasmine, bergamot); c) phenols (such as eugenol – essential oil from cloves, cinnamon, basil, pimento, bay laurel, ylang-ylang, thymol – oil from thyme, oregano, savory, marjoram); and d) terpenes (such as limonene – found in citrus fruit essential oils, pinene – in essential oils from pine, rosemary, eucalyptus, peppermint, cypress, basil, juniper, lavender) (Eslahi et al., 2017; da Silva et al., 2021; Lin et al., 2021). It is understood that terpenoids contain some essential oils that inhibit the growth of *Acinetobacter baumannii* and *Staphylococcus aureus* bacteria. Importantly, such capacity to limit the ability of *S. aureus* is crucial in the formation of biofilms (Adukwu et al., 2016; Patra and Baek, 2016; Silva et al., 2017; Yadav et al., 2015; Subramenium et al., 2015). Various components contained in essential oils may contribute in its capacity to provide synergistic form of antibacterial activity (Lahmar et al., 2017). Similar to this context is the sporicidal activity of cardamom, juniper leaf, and tea tree oils that was reported by Lawrence and Palombo (2009). Specifically, these workers revealed that when the main components of most active oils were individually examined, the sporicidal activity was significantly lower than that of the natural complex compositions of oils. Elsewhere, however, there is the situation of antimicrobial activity revealed by triterpene glycosides isolated from lemon balm leaves particularly against some Gram-negative bacteria species, which compares favorably with those emerging from ampicillin and augmentin, especially against *P. aeruginosa* and *K. pneumoniae* (Abdel-Naime et al., 2019). Many triterpenoid saponins could equally exert significant inhibitory effects on fungi, which are generally mediated by membrane permeability properties that form complexes with sterols. Besides, the actions of extracts from *G. glandulosum* and their isolated flavonoid glycosides would equally be deemed bactericidal (Tagousop et al., 2018).

Challenges and future prospects

Several challenges as well as difficulties appear associated with the use of bioactive additives isolated from plants especially as food additives, such as bioavailability and stability, taste and odor, regulatory requirements, and costs. For instance, in foods and beverage products, there are either polyphenols, essential oils and/or glycosides that occur as part of multicomponent systems, able to interact and affect the product's stability, activity, and bioavailability. For emphasis, the concept of "bioavailability" refers to the rate/extent at which a bioactive compound/nutrient gets absorbed and subsequently avails to the body tissues/organs. In this context, the variability of bioactive compounds to become bioavailable depends on such factors like their chemical structure, food matrix, and interaction with other components (Fernández-Ochoa et al., 2022). Polyphenols would, therefore, become susceptible to degradation/oxidation, which may well influence the shelf life/stability of animal food products, which would deter their capacity when incorporated since their beneficial properties may be lost during processing/storage (Antony and Farid, 2022; Debelo et al., 2020).

The stability of polyphenols would depend on the likes of pH, temperature, as well as processing/storage (Deng et al., 2018). In fact, the solubility and stability of polyphenols appear to be of greater consequence at lower pH values, whereas those of myofibrillar proteins appear so at higher pH values. Therefore, pH of the digestive environment would influence the interaction between polyphenols and myofibrillar proteins, and consequently their bioavailability. Depending on pH level, however, the activity of anthocyanins may well thrive in different chemical forms and color (Cao et al., 2021). Besides, it is important that food producers adhere to labeling requirements stipulated for animal food products. Notwithstanding that countries and regions may have their own laws and regulations regarding the use of glycosides, polyphenols and essential oils as food additives, animal food manufacturers still must adhere to the laws/regulations that assure the product quality and safety. But, to incorporate the isolation of polyphenols, glycosides and essential oils within the animal food production process can be expensive. Moreover, the animal food producers may likely encounter other challenges in the use of polyphenols, which may not necessarily contribute to increases in consumer price. Despite the above-mentioned, the natural food additives offer many potential health benefits, but it must be noted that their use in the animal food production would still have some challenges, which (animal food) producers must be willing to holistically consider.

Table 2. Mechanism types of antimicrobial action and their respective examples specific to polyphenols, essential oils, and glycosides

Mechanism types of antimicrobial action	Polyphenols	Essential oils	Glycosides
Cell wall synthesis inhibition	Epigallocatechin gallate (EGCG) (Higuchi et. al 2024), quercetin (Li et al., 2016)	Tea tree oil (*Melaleuca alternifolia*) (Carson et al., 2006), oregano oil (*Origanum vulgare*) (Sharifi-Rad et al., 2018)	Saponins (Podolak et al., 2010), oleuropein (Zorić and Kosalec, 2022)
Cell membrane depolarization	Catechins (Sirk et al., 2009), ellagic acid (Alfredsson et. al. 2014)	Peppermint oil (*Mentha piperita*) (McKay and Blumberg, 2006), thyme oil (*Thymus vulgaris*) (Marchese et al., 2016)	Digitonin (Hostettmann and Marston, 1995), aescin (Podolak et al., 2010)
Protein synthesis inhibition	Resveratrol (Baur and Sinclair, 2006), quercetin (Li et al., 2016)	Peppermint oil (*Mentha piperita*) (McKay and Blumberg, 2006), tea tree oil (*Melaleuca alternifolia*) (Carson et al., 2006)	Stevioside (Chatsudthipong and Muanprasat, 2009), ginsenosides (Zhang et al., 2012)
Nucleic acid synthesis inhibition	Ellagic acid (Alfredsson et. al. 2014), chlorogenic acid (Sato et al., 2011)	Eucalyptus oil (*Eucalyptus globulus*) (Sadlon and Lamson, 2010), tea tree oil (*Melaleuca alternifolia*) (Carson et al., 2006)	Stevioside (Chatsudthipong and Muanprasat, 2009), ginsenosides (Zhang et al., 2012)
Metabolic pathways inhibition	Resveratrol (Baur and Sinclair, 2006), quercetin (Li et al., 2016)	Thyme oil (*Thymus vulgaris*) (Marchese et al., 2016), lavender oil (*Lavandula angustifolia*) (Cavanagh and Wilkinson, 2002)	Saponins (Podolak et al., 2010), ginsenosides (Zhang et al., 2012)

Concluding remarks

Natural plant substances are promising potential food additives with antioxidant, antimicrobial and functional properties. However, it is necessary to thoroughly investigate the optimal addition of individual compounds to protein-polyphenol, protein-terpene and protein-glycoside matrices, taking into account the desired, expected effect of the given ingredients. Many studies also refer to more complex matrices, which is why the results are not reliable, because the ingredients of meat products, such as lipids or carbohydrate additives, could have influenced the course of chemical reactions and the effect of the compounds. The action of some bioactive compounds requires the use of appropriate carriers, therefore technologies should be developed that enable the use of pure plant ingredients. Many studies refer to myofibrillar proteins obtained from fish, pork or lamb, but little concerns myofibrillar proteins from poultry and beef. Indeed, it is crucial to understand how myofibrillar proteins interact with the specific bioactive ingredients, i.e. biologically active compounds of plant origin, especially their antioxidants/microbial potentials operating as food additives, given the eventual impact to modify the functionality/sensory features of the emergent animal food products.

But, animal food producers must carefully consider a holistic picture regarding the use of bioactive additives isolated from plants. This will help offer a better understanding of their efficacy, especially when bioactive substances are incorporated so as to assure their effectiveness and safety for emergent products, as well as consumer appeal. Further research is needed to better understand how these factors interact, develop food products and dietary interventions that maximize the bioavailability, as well as health benefits of polyphenols in the presence of myofibrillar protein. Additionally, further research is needed on the effects of polyphenols on the human body, as there is much controversy regarding the anti-cancer effect or promoting the development of cancer at too high doses, and there are also some doubts as to the actual effect on the human body, due to the processes occurring in the digestive system.

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