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The use of plant bioactives as a potential antimicrobial in meat and meat products

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Abstract

Infectious diseases and food poisoning from foodborne microorganisms are a major health concern in the modern world. Potential foodborne pathogens cause illness or even death in consumers and this issue highlights the importance of monitoring and prevention. To limit the presence of pathogens in food, natural and synthetic additives are employed in the food systems. Nowadays, natural additives draw the attention of consumers owing to their health endorsing effects and demand for synthetic additives declined due to the safety issues. Meat is the edible flesh of an animal and has high nutritional values. The perishable nature of meat and meat products makes them non-resistant to quality impairments such as microbial deterioration. To enhance the microbial quality of meat and meat products, natural extracts and essential oils from plant sources are employed through different techniques. Among plants, spices and herbs are an excellent source of bioactive compounds particularly polyphenols. Polyphenols are a diverse class of chemicals naturally present in plants and may confer many health benefits along with antimicrobial properties. Polyphenols from herbs and spices are a promising alternative to food safety and conservation. In a nutshell, this paper reviews the possible mechanisms of plant bioactive against microbes and then the use of plant bioactive and essential oils in meat and meat products.

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Introduction

Meat and meat products are sensitive to quality deterioration due to their rich nutritional compounds such as proteins, lipids, vitamins and minerals. Microbial growth and chemical changes are the two main causes of deterioration in meat products (Shah, *et al.*, 2014). As a result of lipid oxidation, undesirable reactions that deteriorate flavor, odor, and color, sensory and textural properties of meat products can occur (Shah *et al.,* 2014). Pathogenic microorganisms can also potentially cause foodborne diseases (Del Nobile *et al.,* 2012). To inhibit lipid oxidation and microbial growth, antioxidants are added to food (José *et al.,* 2014; Granato *et al.,* 2017; Nikmaram *et al.,* 2017; Horita *et al.,* 2018; José Manuel Lorenzo *et al.,* 2018).

To inhibit lipid oxidation, synthetic antioxidants are employed in meat products, however, safety concerns regarding the synthetic antioxidants restricted their use in the food industry (Nikmaram *et al.,* 2017). The meat industry and scientific community paying much attention to plant bioactive due to their antimicrobial, antiviral and antifungal activities (Dalle Zotte *et al.,* 2016; Niaz *et al.,* 2019). Mainly flavonoids, anthocyanins, proanthocyanins, quercetin, carvacrol and thymol, from different plant sources, are responsible for such antimicrobial activity. The presence of these compounds in plants particularly herbs and spices make plants a suitable candidate to be used as a preservative in meat products (Li *et al.,* 2014; José Manuel Lorenzo *et al.,* 2018; Oussalah *et al.,* 2007). Bioactive compounds from different plant sources can inhibit/retard the number of spoilage and pathogenic microorganisms and owing to this property, plant bioactive are suitable replacers of synthetic antioxidants. This review focuses on the use of phytogenic extract to control the microbial activity in meat-based products.

Biosynthesis, classification and distribution of plant polyphenols

Phenolic compounds are ubiquitous in the plant species but their distribution at the plant tissue, cellular and subcellular levels is not uniform. Vacuoles are the location of soluble phenolic whereas insoluble are found in the cell wall of the cell. In the numerous functions of plant physiology for example pigmentation, pathogen, structure, pollination and as well as growth and development they play an important part. Insoluble phenolic plays a regulatory role in the plant growth as well as provide mechanical strength to the cell wall of the plant cell (Vladimir-Knežević *et al.,* 2012). An enhancement of phenylpropanoid metabolism and the number of phenolic compounds can be observed under different environmental factors and stress conditions (Dewick, 2002; Korkina, 2007). Most plant phenolics are derived from trans-cinnamic acid, which is formed from L-phenylalanine by the action of L-phenylalanine ammonia-lyase (PAL). Combine actions of Acylpolymalonate and shikimic acid pathways help in the biosynthesis of Flavonoids. Plants collectively synthesize several thousand known different phenolic compounds and the number of these fully characterised is continually increasing. They can be considered as the most abundant plant secondary metabolites with highly diversified structures, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. The common feature of plant phenolic compounds in the presence of a hydroxy-substituted benzene ring within their structure. They may be classified into different groups as a function of the number of phenol rings contained and the structural elements that bind these rings to one another.

Flavonoids are the subgroup of plant polyphenols with more than 6000 structure identification. Flavonoids are further divided into 6 subgroups i.e. flavonols, anthocyanidins and isoflavonoids as a result of variation in heterocycle structure. In the heterocycle of the flavan frame, flavones are formed due to the presence of a double bond in between carbon 2 and 3. Likewise, flavonols differ only due to the presence of OH group attached at carbon 3 position, whereas there is none double bond in the 3 carbon chain which results in the formation of flavanones. Similar to flavanones, flavanols differ only due to the presence of OH group attached at the

saturated 3 carbon chain. Due to the substitution of phenyl group at 2 position of pyran ring results in the synthesis of anthocyanidins, whereas in term of isoflavonoids this group is attached at 3 position of the pyran ring. (Dewick, 2002; Harborne & Williams, 2000; Middleton *et al.*, 2000). Table 1 summarizes the chemical structures of the most common flavonoids and their botanical sources.

Table 1. Antibacterial action of polyphenols on pathogens.

Antibacterial activity	Site of action	Mechanism of action	Example	References
Interaction with bacterial cell wall	Peptidoglycan	Bound to Peptidoglycan, disturb cells integrity, the resistance of the cell against low osmotic pressure and high ionic strength reduced	Production of grooves from *Pseudomonas aeruginosa* and aggregates from streptococcus mutants when peptidoglycan from gram-negative and gram-positive bacteria bind to EGCG	(Cui *et al.,* 2012)
Interaction with the cell membrane	Bilayer	Membrane integrity and membrane properties irreversibly altered	Increase in cell membrane permeability by tea polyphenols which causes cell membrane disruption and discharge of cellular components in Serratia marcescens	(Yi *et al.,* 2014)
			Disruption and leakage of cellular constituents from cytoplasm membrane of *P. aeruginosa*, *E coli* and others by ferulic acid and gallic acid	(Borges, Ferreira, Saavedra, & Simoes, 2013)
Prevention and control of biofilm	Adhesines	Bacterial attachment inhibited	Adhesion of L. Monocytogenes and *P.aeruginosa* prevented by gallic acid and ferulic acid inhibited the adhesion of *P. aeruginosa* and *S. aureus*	(Borges *et al.,* 2012)
	Polyphenols-Quorum sensing	Inhibition of maturation in biofilm	In *K. pneumoniae*, *Y. enterocolitica*, and *P. aeruginosa* inhibition of exopolysaccharide production by Quercetin	(Gopu, Meena, & Shetty, 2015)
		Inhibition of sporulation by bacteria	Multiplication of *S. aureus* inhibited by ferulic acid	(Borges *et al.,* 2012)
Microbial Enzyme Inhibition and substrate deprivation	Microbial Enzymes	Reduced levels of cyclic -Di-AMP	*B. subtilis* cyclic di-AMP synthase is inhibited by theaflavin-3,3'-digallate, theaflavin-3'-gallate and tannic acid	(Opoku-Temeng & Sintim, 2016)
		Reduced activity of enzymes	Dihydroorotate dehydrogenase, fumarate reductase flavoprotein, dihydrofolate reductase, DNA gyrase and NADH-dependent enoyl ACP-reductase are the target for 19 flavonoids	(Xiao *et al.*, 2014)
Protein regulation	Repression or stimulation of bacterial genes	Down-regulated proteins	Application of cranberry proanthocyanidins on *P. aeruginosa* down-regulated 2 proteins implicated in ATP synthesis, a cytochrome C, hypothetical protein and proteins involved in DNA and RNA synthesis, and acid cycle proteins	(Ulrey *et al.,* 2014)
			By repression of curli genes e.g. csgB and csgA, flavonoid phoretin reduced the biofilm formation by E. coli	(Lee *et al.,* 2011)
		Up-regulated proteins	Application of cranberry proanthocyanidins with *P. aeruginosa* up-regulated proteins related to iron siderophores or cation transporters , proteins involved in amino acids synthesis, proteins involved in response to stress, and a hypothetical protein involved in flavonoid metabolism	(Ulrey *et al.,* 2014)
Metal iron deprivation due to chelating ability	Ionic iron	Iron immobilization	In *P. aeruginosa*, Cranberry proanthocyanidins decreased the content of cytochromes.	(Ulrey *et al.,* 2014)

The hydroxybenzoic and hydroxycinnamic are the two distinctive carbon frameworks of the naturally occurring phenolic acids also known as non-flavonoids. A variety of the compounds is formed due to the changing the number as well as the position of the methoxyl and hydroxyl group on the benzene ring, although the basis frame remains the same. Another small group of non-flavonoids is the stilbenes which are found in a variety of plant sources. Stilbenes exist as stereoisomers and naturally occurring stilbenes are overwhelmingly present in the *trans* form. Similarly, another small group of non-flavonoids phenolics is the lignans which are formed as a result of the coupling of two phenylpropanoid units as shown in Fig. 1. Lignans have minor glycoside derivatives while remaining exist in free form (Willför *et al.,* 2006).

Possible antimicrobial mechanism

It's unclear how polyphenols inhibit or kill bacteria. The polyphenols interact with other non-specified factors such as hydrophobic effects, lipophilic and hydrogen forces including the formation of covalent

bonds following enzymes, adhesins and transporter proteins related to cell envelope (Cowan, 1999; Kumar & Pandey, 2013). The antibacterial and antimicrobial capacity of polyphenols is associated with their ability to chelate or resemble iron and it is considered essential for the durability of all bacterias (Field & Lettinga, 1992).). Possible mechanism of polyphenols against pathogens is given in table 1.

Interaction of polyphenols with the microbial cell wall

The Gram-positive and Gram-negative bacterial cell walls are non-identical from each other. The cell wall of Gram-negative bacteria consists of an outer membrane and a thin layer known as peptidoglycan. The outermost membrane of cells consists of proteins and bilayer phospholipids in addition to lipid-containing polysaccharides. The cell wall of Gram-negative bacteria is composed of lipoteichoic acid, peptidoglycan and lacks an outer membrane (Brown, *et al.,* 2015). In both Gram-negative and Gram-positive bacteria's cell wall plays a pivotal role in protecting the osmotic regulation of cells. The toleration of cells to lowered osmotic pressure and elevated ionic strength is decreased due to damaged cell walls. The peptidoglycan is vital for the viability of bacteria and it is also very important for interacting antibiotics. Many scientists have evaluated the activity of polyphenols to assemble with the cell wall of bacteria and polyphenols susceptibility of Gram-positive bacteria is less as compared to Gram-negative bacteria. This is due to the change in the composition of cell walls. The Gram-negative bacteria's hydrophilic membrane consists of lipid-containing polysaccharides (Nohynek *et al.,* 2006) and inhibits the interaction of polyphenols with peptidoglycan (Cui *et al.,* 2012).

Interaction of polyphenols with the microbial cell membrane

Many scientists have reported about polyphenols interaction bacterial membrane such as catechins (Cho, Schiller, Kahng, & Oh, 2007; Ikigai *et al.,* 1993; Matsumoto *et al.,* 2012). It has also been analyzed that there is a positive interaction of polyphenols with phospholipids and proteins. In both Gram-negative and Gram-positive bacteria disruption of peptidoglycan and elevated permeability of the membrane is caused by interaction with membrane related proteins (Nazzaro *et al.,* 2013).

Biofilm inhibition

During handling and slaughtering of meat; pathogenic and spoilage causing microorganisms can contact with the meat surfaces and causes deterioration leading to the formation of biofilms. In meat industries, spoilage and pathogenic bacteria form biofilms which can be a continuous source of contaminated meat products leading to serious health issues (Van Houdt & Michiels, 2010). The formation of biofilms is dependent on four steps; (i) Attachment of bacteria to rigid surfaces which is reversible (ii) irreversible binding (attachment of bacteria to surfaces having copolymer structures (iii) multiplication (maturation, formation of extracellular copolymer substances and (iv) Scattering (dispersion of microorganisms from various biofilms) (Rijnaarts *et al.,* 1995; Katsikogianni & Missirlis, 2004; Van Houdt & Michiels, 2005; Myszka & Czaczyk, 2011). Various studies have analyzed that polyphenols depicted anti-biofilm and antimicrobial properties.

Bacterial enzymes Inhibition and substrate deprivation

Various studies reported that polyphenolic compounds can inhibit cyclic AMP synthase, the enzyme which is required for the catalysis and biosynthesis of cyclic di-AMP; a vital signaling protein that is required to control various functions particularly homeostasis (Opoku-Temeng & Sintim, 2016), bacterial cell wall modulation and its synthesis (Huynh *et al.,* 2015; Sureka *et al.,* 2014; Witte *et al.,* 2013).

Protein regulation

The synthesis of protein may be a primary cause for the antimicrobial activity of polyphenols. (Ulrey *et al.,* 2014) suggested *P. aeruginosa* treatment with downrigger 2 protein having proanthocyanin activity is embroiled in DNA and RNA synthesis, cytochrome

C and PA 2482 mainly debatable protein and subunits of acetyl fumarase and acetyl-CoA. However, they have also noticed the up-regulation of many other proteins as well. Thus, twelve proteins which are related to cation transporter or iron siderophil (PvdN, PhuS and PchD), five proteins involved in the synthesis of amino acids (PA2044, HutG and PA0335), proteins involved in response to stress (SodM and OsmC) and a protein (PA3450) which is upregulated in the metabolism of flavonoids.

Iron chelating ability and iron deprivation

Iron is a pivotal mineral for the spoilage and growth of foodborne microorganisms except for LAB. Meat is a major medium for the growth of spoilage and deteriorating bacteria because it contains significant hematin (heme) proteins mainly haemoglobin and myoglobin and non-hematic proteins particularly ferritin and transferrin are good sources of iron for the spoilage causing bacterial growth. Non-enzymatic and enzymatic degradation of proteins mainly heme can also help bacteria to get maximum iron. Thus, lowering the obtainable iron ion levels may help in inhibiting microbial and bacterial growth (C.-M. Kim & Shin, 2009; Moon *et al.,* 2013; Thompson *et al.,* 2012).

Antimicrobial effect of plant extracts

There are many studies on the use of extracts and especially essential oils from different plant sources such as ginger, cinnamon, garlic, rosemary, oregano, basil, cloves, marjoram, turmeric and sage to determine the antimicrobial activity on meat products (Del Nobile *et al.,* 2012).

(Agrimonti *et al.,* 2019) evaluated the antimicrobial activity of essential oils (thyme, oregano) containing cellulosic pads against psychrophilic bacteria in minced beef and found these pads effective against particular meat species such as *Lactococcus lactis, pseudomonas spp* and *Enterococcus faecalis*. Additionally, essential oil containing pads were effective against some other foodborne pathogens such as *S. enterica, C. jejuni* and *S. aureus*. Moreover, (Zhang *et al.,* 2016) explored the effect of bioactive from Rose in dry fermented sausages. Rose extract reduced the formation of biogenic amines which are associated with microbial spoilage. One study scrutinized the bactericidal effect of *Syzygium antisepticum* extract in cooked chicken against *S. aureus*. At this concentration level, extract inhibited the growth for 16 hours in the case of *S. aureus* while in the case of MRSA, extract inhibited the growth for 8 hours. Another study evaluated the shelflife of raw chicken meat by adding dried cloves, mustard, cinnamon and oregano alone and in combination (Abdulla *et al.,* 2016). They found that extracts significantly inhibited the total viable count, LAB, pseudomonas sp and Enterobacteriaceae. By assessing the antimicrobial effect of oregano extract in sheep burgers stored in MAP. Although microbial count increased with the passage time, oregano extract containing samples slightly improved the microbial quality of sheep burgers compared to control. (Zhang *et al.,* 2016) explored that the progression of *Bacillus subtilis, Neisseria gonorrhoeae, Escherichia coli, Streptococcus faecalis, Staphylococcus aureus* and *Pseudomonas* in sausages retarded by the Ziziphus leaves extracts and limit the viable count. In fresh chicken meat, microbial progression was significantly effective due to spice extracts (cloves, rosemary and their blends) (Zhang *et al.,* 2016). (Nejad *et al.,* 2014) explored that the development of S. aureus in hamburgers was efficiently reduced by 1ml garlic aqueous extract. (Alkass *et al.,* 2013) pointed out that the lipolytic, coliform, psychrophilic and proteolytic bacteria activity was highly affected due to ginger and rosemary extracts and their blends with sodium lactate. (Uçak *et al.,* 2011) found that in fish burger microbial activity can be controlled by rosemary extract in combination with vacuum wrapping. However the retardation of *L.monocytogenes* contaminations in cooked chicken meat through ethanolic leaf extract of *Rhodomyrtus tomentosa*. (Olaimat & Holley, 2016) reported that L.monocytogenes and lactic acid bacteria sustainability controlled by allylisothiocyanate (AITC) also lowered the progression of potential spoilage

bacteria on cooked chicken breasts. (Jalosinska & Wilczak, 2009) explicated that rosemary extract containing sample had the highest antimicrobial activity and rosemary, lovage and cranberry extracts subdued the growth of microbes in meatballs.

The numbers of *E. coli* O157:H7, *L. monocytogenes, Aeromonas hydrophila* and *Salmonella Typhimurium* were significantly decreased in cooked beef by pine bark and 1.0% grape seed extracts (Ahn, Grün, & Mustapha, 2007). (S. Kim & Fung, 2004) expounded that in-ground beef *L. monocytogenes* and *A. enterica* serotype enteritidis activity reduced to some extent by arrowroot tea extract. (Careaga *et al.,* 2003) found that 3mL/100g Capsicum extract dosage was acceptable for bactericidal effect against *P. aeruginosa* and 1.5ml/100g extract was suitable to reduced the *S.typhimurium* activity in fresh minced beef. (Olaimat, Fang, & Holley, 2014; Olaimat & Holley, 2015) explored that the sustainability of *C. jejuni* and *Salmonella* reduced on fresh, Vaccum packed chicken breasts stored at 4°C for 21 days by preparation of κ-carrageenan/chitosan coatings with 1% (v/v) acetic acid and 50ml/g AITC or 250-300mg/g oriental mustard extract. One study reported a decrease in mesophilic counts in minced chicken meat treated with clove extract at 0.15 percent level and stored at refrigeration temperature. The analysis of the counts of control and clove treated samples using t-test revealed a highly significant ($p<0.01$) reduction in counts in clove treated samples throughout the storage period. Extended shelf life has been observed in buffalo meat mince treated with clove essential oil stored at refrigeration temperature as compared to control samples. One study describes, on bologna slices species of L.monocytogenes cells reduced to an imperceptible level (<1.6 log10 CFU/g) at 4°C after 52 days by a polymeric film containing the oriental mustard extract. (Higginbotham *et al.,* 2014) explored the anti-microbial effect of hibiscus flower in beef hot dogs and concluded that higher *L. monocytogens* prevention may be achieved by employing the higher concentrations of hibiscus flower extract. Similarly, (Perumalla *et al.,* 2013) expounded that green tea and grape seed extract are effective against the food spoilage bacteria and are a good replacer of chemical preservative when combined with the heat.

(Dussault *et al.,* 2014) found a significant decrease in the growth of pathogens by the application of essential oils (oregano and cinnamon) in ham. Moreover, (Park, Park, & Yoon, 2014) described that the growth of Clostridium perfringens might be lowered by combining rooibos and potassium lactate+ sodium diacetate in jokbal.

Conclusion

Due to improper hygiene and food safety, many people die every year. Foodborne pathogens are responsible for several outbreaks of foodborne diseases. Improper handling and storage of meat and meat products might be a major reason for microbial deterioration, which further affects the quality and safety of meat. Bioactive compounds derived from plants are found to be effective natural antimicrobial in meat and meat products. Among plant extracts and essential oils, phenolic compounds are the key components that are responsible for the antimicrobial properties. The use of these compounds in the meat industry should be encouraged due to their capability to retard/inhibit the growth of foodborne pathogens. Despite having antimicrobial properties, phenolic compounds provide health endorsing properties.

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