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Antimicrobial edible films and coatings

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Abstract
Edible coatings are food grade suspensions which may be delivered by spraying, spreading, or dipping, which upon drying form a clear thin layer over the food surface. Coatings are a particular form of films directly applied to the surface of materials and are regarded as part of the final product. On the other hand, edible films are obtained from food grade filmogenic suspensions that are usually cast over an inert surface, which after drying can be placed in contact with food surfaces. Films can form pouches, wraps, capsules, bags, or casings through further processing and one of the main differences between films and coatings is their thickness. When antimicrobial agents such as benzoic acid, sorbic acid, propionic acid, lactic acid, nisin, and lysozyme have been incorporated into edible films, such films retarded surface growth of bacteria, yeasts, and molds on a wide range of products, including meats and cheeses. Various antimicrobial edible films have been developed to minimize growth of spoilage and pathogenic microorganisms, including Listeria monocytogenes, which may contaminate the surface of cooked ready-to-eat foods after processing. Here, we review the various types of protein-based (wheat gluten, collagen, corn zein, soy, casein, and whey protein), polysaccharide-based (cellulose, chitosan, alginate, starch, pectin, and dextrin), and lipid-based (waxes, acylglycerols, and fatty acids) edible films and a wide range of antimicrobial agents that have been or could potentially be incorporated into such films during manufacture to enhance the safety and shelf life of ready-to-eat foods.

Keywords: edible, antimicrobial, coatings, films, sorbic acid, benzoic acid, propionic acid

Introduction

Edible coatings are food grade suspensions which may be delivered by spraying, spreading, or dipping, which upon drying form a clear thin layer over the food surface. Coatings are a particular form of films directly applied to the surface of materials and are regarded as part of the final product. On the other hand, edible films are obtained from food grade filmogenic suspensions that are usually cast over an inert surface, which after drying can be placed in contact with food surfaces. Films can form pouches, wraps, capsules, bags, or casings through further processing and one of the main differences between films and coatings is their thickness. The use of films in foods dates back to the 12th century in China where waxes were used to coat citric fruits to retard water loss, whereas the first edible film used for food preservation was made in the 15th century from soymilk (Yuba) in Japan. In England lard or fats were used as coating to prolong shelf life of meat products in the 16th century and in Europe; this process was known as "larding". In the nineteenth century, a US patent was issued in relation to preservation of meat products by gelatin coatings. Edible films and coatings (EFC) are an alternative to extend the shelf life of AOF by acting as barriers to water vapor, oxygen, and carbon dioxide and as carriers of substances to inhibit pathogenic and spoilage microorganisms. Natural antimicrobial agents may be incorporated into the corresponding suspensions, adding functionality to edible films and coatings, leading to the antimicrobial edible films and coatings (AEFC) obtaining. There is increased interest in development and use of AEFC to preserve meat quality for longer shelf life periods while maintaining food safety, which is based on consumers demand for natural and safe products. Industry is concerned about these issues, while keeping competitive production costs. Other key issues are sustainability through the use of biodegradable packaging materials and applications of by-products from the food industry that can generate added value. Due to similar properties of edible films and coatings this review discusses characteristics of both types of coverings applied to meat products. This work focuses on a critical discussion of issues raised by recent research findings on the effectiveness of antimicrobial films and coatings and their potential application to enhance safety and quality of meat products.

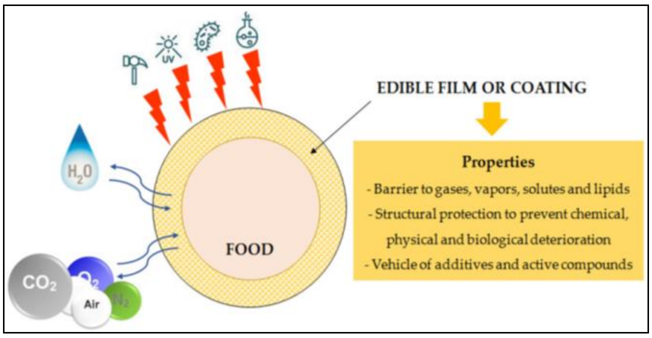

Fig 1: Properties of edible film or coating

Definition and historical background of edible film

Edible Ž films or coatings are deŽ need as continuous matrices that can be prepared from proteins, polysaccharides, and lipids. Yuba, the Ž rest free-standing edible Ž lm, was developed in Japan from soymilk during the 15th century and was used for food preservation. Edible coatings for food products date back even further; during the 12th century in China, waxes were applied to oranges and lemons to retard water loss. During the 16th century, food products were coated with fat (e.g., lard) to control moisture loss. Hot-melt paraffinŽ n waxes have been used to coat citrus fruits in the United States since the 1930s, and carnauba wax and oil-in-water emulsions have been used for coating fresh fruits and vegetables since the 1950s. Currently, edible films and coatings are used in various applications, including casings for sausages and chocolate coatings for nuts and fruits.

Composition and Properties of Protein-Based Films and Coatings

Film-forming proteins are derived from animals (casein, whey protein concentrate and isolate, collagen, gelatin, and egg albumin) or plant sources (corn, soybean, wheat, cottonseed, peanut, and rice). Protein-based films adhere well to the meat hydrophilic surfaces and provide barrier for oxygen and carbon dioxide but do not resist water diffusion. Plasticizers, such as polyethylene glycol or glycerol, are added to improve flexibility of the protein network, whereas water permeability can be overcome by adding hydrophobic materials such as beeswax or oils like oleic that can affect films properties such as crystallinity, hydrophobicity, surface charge, and molecular size, improving films characteristics and their application. Despite their advantages, protein films may be susceptible to proteolytic enzymes present in meat products or allergenic protein fractions may cause adverse reactions to susceptible people.

Composition and Properties of Polysaccharides-Based Film and Coatings

Polysaccharide coatings are generally poor moisture barriers, but they have selective permeability to O_2 and CO_2 and resistance to fats and oils. Polysaccharide films can be made of cellulose, starch (native and modified), pectins, seaweed extracts (alginates, carrageenan, and agar), gums (acacia, tragacanth, and guar), pullulan, and chitosan. These compounds impart hardness, crispness, compactness, viscosity, adhesiveness, and gel-forming ability to a variety of films. Polysaccharide films and coatings can be used to extend the shelf life of muscle foods by preventing dehydration, oxidative rancidity, and surface browning. When applied to wrapped meat products and exposed to smoke and steam, the polysaccharide film actually dissolves and becomes integrated into the meat surface resulting in higher yields, improved structure and texture, and reduced moisture loss.

Common Antimicrobials Used in EFC

Incorporation of antimicrobial compounds into EFC as an alternative to their direct application onto the meat surface has the advantage of gradual release of the antimicrobial compound from the AEFC leading to a reduction of added antimicrobial and to reduced sensory changes. Antimicrobial compounds within AEFC are less exposed to interaction with meat surface components than those added directly to the surface and thus maintaining their activity.

The characteristics and mode of action of most common antimicrobials used to promote meat safety are described below.

Organic Acids

The antimicrobial effect of organic acids depends on concentration of undissociated form, which can penetrate the bacterial cell membrane. Inside the cell, their dissociation leads to interference with membrane transport and disruption of proton motive force. Organic acids incorporated into EFC include lactate and acetate, propionate and *p*-aminobenzoic acid WPI coatings added with malic acid, nisin, and grape seed extract applied on turkey frankfurters decreased to 2.3 log CFU/g of *L. monocytogenes* and 5 log CFU/g *S. typhimurium* after 28 d of storage at 4°C. Zein based AEFC using calcium propionate combined with nisin, reduced up to 5 log CFU/g of *L. monocytogenes* after 14 d at 4°C, when used to coat chicken breast. Sodium lactate combined with other commercial antimicrobials reduced to 3.5 log/cm^2 of this pathogen when roasted turkey was stored at 4°C for 8 weeks. Thus, organic acids, especially when acting combined with other antimicrobial agents, have an important role in maintaining microbiological quality of meat and meat products.

Essential Oils and Plant Extracts

Essential oils are complex mixtures of volatile compounds obtained from plants, which mainly include terpenes, terpenoids, and aliphatic chemicals, all characterized by low molecular weight. Oils containing phenols such as thymol, carvacrol, and eugenol exhibit the highest activity against all kind of microorganisms. Essential oils usually show higher antibacterial activity than mixtures of their major antimicrobial components, suggesting that minor components are critical for enhanced activity. The antimicrobial mechanism is attributed to the disturbance of the cytoplasmic membrane disrupting the proton motive force; active transport and coagulation of cell contents may occur. Direct incorporation of essential oils in the formulation of AEFC applied to meat products is expected to reduce bacterial population but may alter their sensory characteristics. Microencapsulation of essential oils or their ingredients may be an alternative to protect them from interaction with environmental factors, avoiding their oxidation or volatilization while exerting their antimicrobial effect. Moreover, encapsulation increases the oil solubility in water, prevents its release at an undesired stage, and makes it easier to handle. Essential oils or their constituents that may be incorporated in AEFC on AOF include those extracted from lemongrass, oregano, pimento, thyme, or cinnamon. Oregano essential oil has been the most commonly reported in recent years including a 1.5% extract (v/v), successfully used to reduce total viable count by 2 log CFU/g of cold smoked sardine covered with an AEFC after 20 d storage at 5°C, whereas at 1.9% it achieved *L. monocytogenes* population reduction by 2.4 log CFU/g after 28 d, at 4°C in wrapped cold smoked salmon. Oregano essential oil combined with thyme extract, was incorporated into a film placed on top and bottom of fresh ground beef patties reducing *Pseudomonas* spp. and coliforms populations, whereas mixed with pimento essential oil, the

Films covering beef muscle slices reduced to 1 log of *E. coli* O157:H7 after 7 d of storage at 4°C. Grapefruit seed extract (GSE) incorporated into AEFC was found to inhibit *E. coli* O157:H7 and *L. monocytogenes* from pork loins, bacon, and salmon. However, some commercial GSE is adulterated with synthetic preservatives such as benzalkonium and benzethonium chlorides, which are solely responsible for the antimicrobial activity of GSE. These compounds show toxicity and allergenicity to humans, and it is unlikely that they are formed during any extraction and/or processing of grapefruit seeds and pulp.

Bacteriocins

Bacteriocins from lactic acid bacteria are peptides produced by bacteria that inhibit or kill other related and unrelated microorganisms. These agents are generally heat-stable, apparently hypoallergenic and readily degraded by proteolytic enzymes in the human intestinal tract. Class I bacteriocins, such as nisin, bind to plasma membranes via nonspecific electrostatic interactions and have a dual mode of action. The antibacterial activity results from pore formation in the bacterial plasma membrane, leading to dissipation of the transmembrane potential and vital solute gradients. The high efficiency of pore formation is the result of a second mechanism involving the cell wall precursor Lipid II which increases the affinity of nisin for the membrane, stabilizes a transmembrane orientation of nisin, and forms and integral part of the nisin pore. The pore structure involves a complex made up of four lipid II and 8 nisin molecules, which interferes with peptidoglycan biosynthesis. Other bacteriocins such as pediocin have been widely studied in food systems, but nisin remains the only one approved by European Union (EU) and the USA where it enjoys GRAS status. The effect of nisin incorporation into AEFC is the most studied, either to protect beef and turkey frankfurters, or turkey bologna against *L. monocytogenes* but pediocin has also been tested.

Proteins

Lysozyme is a naturally produced enzyme active against gram-positive bacteria, by hydrolyzing N-glycosidic bonds connecting N-acetyl muramic acid with the fourth carbon atom of N-acetyl glucosamine of the peptidoglycan molecule in the cell wall. This antimicrobial has been formulated in whey protein isolate (WPI) films and tested for its diffusivity and antimicrobial effect on salmon slices and also tested in ground beef patties using zein films.

Chitosan

Chitosan is a linear polysaccharide composed of randomly distributed *β*-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine. Chitosan is believed to chelate certain ions from the lipopolysaccharide (LPS) layer of the outer membrane of bacteria or to exhibit electrostatic interactions among its groups and the negative charges of microbial cell membrane. In both cases cell permeability increases releasing key cellular components of bacteria. The antimicrobial action of chitosan is influenced by type of chitosan, degree of polymerization, and environmental conditions. Chitosan coatings act as barrier against oxygen transfer leading to growth inhibition of aerobic bacteria. In addition to the functionality of chitosan as polymeric material and antimicrobial agent it has been used as coating and wrapper in salami and as film and coating combined With lauric arginate and nisin to reduce *L. monocytogenes* population in sliced turkey deli meat also in seafood and fish.

Lauric Arginate

Lauric arginate (LAE) is a food-grade cationic surfactant that is highly active against a wide range of food pathogens and spoilage microorganisms including bacteria, yeasts, and molds. It is obtained through the reaction of L-arginine, hydrochloric acid, ethanol, thionyl chloride, sodium hydroxide, lauryl chloride, and deionized water. LAE affects cells viability by disturbing membrane potential and causing structural changes, although no disruption of cells is detected. In gram-negative cells, LAE alter both the cytoplasm membrane and the external membrane, while in gram-positive cells, alterations were observed in the cell membrane and in the cytoplasm. However, in both cases, cells remained intact and cell lysis is not observed. LAE is nontoxic and is metabolized to naturally occurring amino acids, mainly arginine and ornithine, after consumption. Effectiveness of LAE, alone or in combination with other antimicrobials, has been tested against *L. monocytogenes*, *S. enterica*, and *L. innocua* in cooked ham and sliced turkey deli meat producing 2 log reductions in all cases

Antimicrobials used in edible films and Coatings

Edible films can serve as carriers for a wide range of food additives, including various antimicrobials that can extend product shelf life and reduce the risk of pathogen growth on food surfaces. Some of the more commonly used preservatives and antimicrobials include benzoates, propionates, sorbates, parabens, acidifying agents (e.g., acetic and lactic acids), curing agents (e.g., sodium chloride and sodium nitrite), bacteriocins, and natural preservatives (e.g., essential oils, lysozyme, liquid smoke). These agents are discussed here in the context of their use or potential use in protein-, polysaccharide-, and lipid-based edible films.

Benzoic acid and sodium benzoate

Sodium benzoate was among the rest chemical preservatives permitted in foods by the Food and Drug Administration. In the United States, benzoic acid and sodium benzoate are generally regarded as safe preservatives at levels up to 0.1%. Although typically used as a mold and yeast inhibitor, sodium benzoate and benzoic acid are also inhibitory to pathogenic and psychrotrophic spoilage bacteria. Sodium benzoate is one of the most commonly used antimicrobials in edible. lms because it is soluble in most. Lm solutions and remains active after. Lm preparation.The antimicrobial activity of sodium benzoate is related to pH. Like many other food antimicrobials, sodium benzoate (pKa 5 4.20) is most effective in its undissociated form; 60% of the compound is undissociated at pH 4.0. Therefore, methycellulose, chitosan, and collagen. Films, all of which have a relatively low pH, are good candidates for this antimicrobial.

Sorbic acid and potassium sorbate

Sorbic acid is a straight chain a,b-unsaturated monocarboxylic acid with the carboxyl group reacting to form calcium, sodium, or potassium salts and esters. Potassium sorbate, the commonly used salt of sorbic acid, is highly soluble in water (58.2% at 208C). Increased antimicrobial activity of potassium sorbate at low pH has been reported for a wide range of microorganisms. Therefore, edible films containing potassium sorbate are typically most effective at pH values less than 6.0. Sorbic acid salts have been among the most studied antimicrobial agents in carbohydrate- and protein-based edible films such as methylcellulose, whey protein isolate (WPI), and chitosan because the sorbates are widely used and remain chemically active in the film matrix. The carboxyl group (the active site in sorbates) forms hydrogen bonds with carbohydrate or protein chains in films. Edible films containing sorbates have been tested against a wide variety of microorganisms (i.e., spoilage bacteria, pathogenic bacteria, yeast and molds) in laboratory media using film disc diffusion assays.

Propionic acid

Propionic acid or its salts are commonly used food preservatives because of their wide spectrum of activity. Propionic acid, a monocarboxylic acid, is produced by *Propioni bacterium freudenreichii* subsp. *shermanii.* Swiss cheese contains up to 1% propionic acid from the growth of propionibacteria, which gives it a characteristic favor and prevents mold growth. Antimicrobial activity of the propionates is again pH dependent, with the undissociated form showing 45 times more inhibitory activity than the dissociated form.

Advantages of Edible Microbial Coating

- Shelf life enhancement of food product
- Prevention of microbial spoilage
- Better keeping and product quality
- Controlling pollution by limiting the use of plastic and other non-biodegradable packaging material
- Shelf sterilizing properties of the food
- Preserving oxidant activity
- Reducing firmness loss by 25 %
- Reducing weight loss in the range 14-24 %
- Prevention of food borne infection

Conclusion

Edible microbial coating is a modern packaging technique that helps to prevent microbial spoilage. It helps to increase the shelf life of the food item and also helps to preserve oxidative activity. It is light in weight so it is easily transported. Edible microbial coating also helps to controlling food borne infection

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