

Incorporation of essential oils (EOs) and nanoparticles (NPs) into active packaging systems in meat and meat products: A review

Mina Kargozari ¹, Hassan Hamedei ^{2*}

¹ Department of Food Science, Islamic Azad University, Tehran-North branch, Tehran, Iran

² Department of Food Safety and Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran

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ABSTRACT

Novel concepts of smart/intelligent, active and eco-friendly food packaging systems, are getting much more attention these days. There have been new functionalities ascribed to the packaging, mostly derived from recent consumer's request for organic and clean-label –high-quality products. This paper throws light on the current advances in antimicrobial active packaging of fresh meat and meat-based products. Among the classes of antimicrobials proposed and tested with remarkable antimicrobial power against microorganisms involved in meat spoilage and meat-borne diseases, we focused on plant-derived essential oils (EOs) and nanoparticles (NPs) as they were attractive meat protecting agents according to the literature review, we have made. The various technologies and methods for incorporating antimicrobial compounds into the package including embedding for controlled release, immobilization, and layer-by-layer deposition, photografting and their feasible approach for active meat packaging are surveyed and scrutinized.

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1. Introduction

There has been a crucial paradigm change in packaging from passive to active as it appears a passive component no longer, is nowadays considered as an active part interacting with the external environment and with the food inside as well (1, 2). The phenomenon could be established by fusing an active agent within a packaging material or at the product contact surface (3). The incorporation of the active agent into the packaging matrix is the most dominant method used to develop active packaging (4). The active component of these antimicrobial systems may act either by gradually releasing into the circumambient atmosphere or by absorbing the deteriorative compounds (5). The active agents would be categorized as direct additives, as the functional agent is intended to contribute to the food product's ingredients' profile (6). It is more obvious, for instance, in "sous-vide" cooked products when direct contact of the packaging materials to the food surface is necessary due to the non-volatile nature of the active agents (7). While effective, such migratory active packaging technologies, non-migratory

active packaging techniques also present remedy strategies for food preservations without the involvement of food additives. In this case, a covalent bond exists between the active agent and the packaging material so that it imparts activity without migrating to the food structure (6, 8). Antimicrobial active packaging (AAP) which falls under the family of active packaging is indeed packaging system containing antimicrobial agents (AAs) (9). Traditional direct applying of the AAs onto food surfaces (e.g. dipping, spraying or pulverization) may result in the taste changes due to immoderate amounts of the active components. Early evaporation of active agents and inactivation or denaturation of them by food ingredients and also an expeditious migration into the food mass may occur using direct application techniques (7, 8). Whereas, the slow migration of the substances away from the surface of packaging material may have a privilege of maintaining the AA at high concentration level over a long period (9, 10). Furthermore; upon subsequent controlled release of AA from the package, the use of AAP can progressively be effective during impending phases of food transportation, storage, and handling, and even once the

* Corresponding author: Department of Food Safety and Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran.
E-mail address: hamedih@ut.ac.ir (Hassan Hamedei)

package is opened, the antimicrobial layer may permanently be active (7, 11). Meat and meat-based products are suffering from a significant level of spoilage (as high as 40% for raw meat) taking place every year (12, 13). Since the microbial contamination of fresh meats comes about primarily at the surface, due to post-processing manipulation, the AAP would be a highly efficient form of active packaging in this case (11, 14, 15). The use of AAs in meat and meat-based products has been approved in 21 Code of Federal Regulations for applying in meat, poultry, and egg products as food additives. For the European Union (EU), the use of food additives in meat preparations is harmonized across the Commission Regulation (EU) No. 601/2014 (16). This paper reviews much of the scientific literature on the diversity of packaging systems and technologies presently accessible for muscle foods, particularly fresh and cooked meats and meat products. In summary three principal classes of antimicrobial films are present: (i) sachet attached to the package containing the active agents, widely used in meat packaging systems; (ii) direct dispersion of the AAs into the packaging media and (iii) coating of the packaging with a substance that acts indeed as a carrier for the AAs. Among these systems based on active meat packaging, items (ii) and (iii) are reviewed in this paper. However, the addition of sachets/pads containing AAs to meat products' packages is well reviewed extensively by Véronique (8). The features such as oxygen-scavenging systems which could structurally take the form of a sachet, film, etc. and possess indirect antimicrobial properties against aerobic bacteria and fungi have also been excluded from this study. More comprehensive enlightenments and details regarding similar concepts could be achieved from other review articles (8, 14).

2. Basic meat packaging materials

It is stated that the polymer types utilized as contact layers for refrigerated and frozen fresh meats are 79% poly(styrene) (PS), 38% poly (vinyl chloride) (PVC) and poly (vinylidene chloride) (PVdC) and thermoplastic polyolefins (POFs): 13% poly(propylene) (PP) and 8% polyethylene (PE). Modified atmosphere packaging (MAP)-based systems may exploit thermoformed trays made from unplasticized PVC/PE, poly (ethylene terephthalate) (PET)/PE, PS/ethylene vinyl alcohol (EVOH)/PE, or PET/ethylene vinyl acetate (EVAC)/PE while preformed base trays are often made from PET, PP, or unplasticized PVC/PE. Lidding sheets are often PVdC coated PET/PE; PVdC coated PP/PE, or poly(amide) (PA)/PE. Flow wrap films may be PA/PE, PA/ionomer, or PA/EVAC/PE (17-19). Moreover; plasticizers could take part in the structure of the active films in order to provide flexibility and give the desired mechanical properties to the film. Polymers originated from renewable resources has received growing attention over the past two decades due to consumer apprehension toward natural food products and increasing concern of more environmentally friendly packaging (20). Based on their origin, biopolymers can be classified into three principal categories: (i) natural biopolymers on the basis of renewable

feedstocks such as plant carbohydrate (e.g. starch and cellulose) and animal or plant-originated proteins (e.g. gelatin, whey protein, zein); (ii) biodegradable synthesized polymers manufactured from petrochemical resources [e.g. poly(l-lactide) (PLA), poly(glycolic acid) (PGA), poly(ϵ -caprolactone) (PCL), poly(vinyl alcohol) (PVA)]; (iii) biopolymers fabricated by means of biotechnology (e.g. microbial polyesters, such as poly(hydroxyalkanoates) (PHAs) and microbial polysaccharides, such as pullulan and curdlan) (21, 22). Another study was conducted by Sivarajan et al. (23) to investigate the effect of edible starch films assimilated with clove and cinnamon on the shelf life of white shrimps (*Litopenaeus vannamei*) stored at two different conditions (10 and 4 °C). Shrimp samples wrapped with spice-grafted edible films had lower bacterial counts and consecutive extended shelf lives. The antimicrobial function could be achieved by adding AAs into the packaging system and/or using antimicrobial polymers e.g. chitosan that has inherent antibacterial activity (18, 24). Siripatrawan and Noipha (25) developed the active film from chitosan incorporating green-tea-extract for pork sausage preservation. The antimicrobial activity of chitosan and its observed synergism with green-tea-extract was explained. It was postulated that total aerobic counts (TAC) and yeasts-molds counts (YMC) in pork sausages wrapped with green-tea-extract chitosan film were lower than those wrapped with chitosan film, but there was no significant difference between the referred samples regarding LAB population. Sánchez-Ortega et al. (26) briefly reviewed edible films and coating types associated with fresh and processed meats' packaging. New Gem™, which contains spices used to enhance ham glaze and Coffi™, made from collagen nettings used to wrap boneless meat products are two commercially available edible films for meat concerns.

3. AAs for use in AP of meat and meat products

The classes of AAs proposed and tested for meat packaging concerns, range from natural biopolymers (chitosan), organic acids or their relevant acid anhydrides, alcohols, bacteriocins (nisin and pediocin), chelators, and enzymes (lysozyme) (Table 1) (11, 27). The incorporation of lactic acid bacteria (LAB) into biopolymer films also arises a fascinating novel approach (28). Trinetta et al. (29) monitored the effectiveness of sakacin A, a bacteriocin produced by *Lactobacillus sakei* DSMZ 6333, against epidemic strains of *L. monocytogenes* isolated from foodborne outbreaks. When artificially contaminated surfaces of turkey breast were treated with the sakacin A-containing (1 mg/cm²) pullulan films, *L. monocytogenes* counts were decreased 3 log-cycles after 3 weeks at 4°C. Various combinations of such antimicrobials are also exploited by fusing into packaging materials in order to determine the synergistic/antagonistic effects (30-33). In a recent article by Ünalán et al. (34), the developed Zein films containing 700 µg cm⁻² lysozyme and 300 µg cm⁻² disodium ethylenediamine tetraacetic acid (Na₂EDTA) exhibited antimicrobial function on *L. monocytogenes*, *E. coli* O157: H7,

and *S. Typhimurium*. The applied lysozyme and Na₂EDTA into the films on beef patties remarkably reduced total viable count (TVC) and total coliform counts after 5 days of storage in comparison to those of control patties ($p < 0.05$) (34). In a study by Pattanayaiying et al. (35), the individual or combined effect of lauric-arginate and nisin loaded pullulan films were examined in vitro (plate overlay assay) and in situ (applied to raw turkey breast, deli ham, and raw beef). The qualitative antimicrobial activity of the active films revealed a synergistic effect of the combination of lauric-arginate and the bacteriocin. *S. Typhimurium* and *S. Enteritidis* on raw turkey breast slices wrapped with a film loaded with lauric-arginate or the combination were reduced throughout the experiment. Pullulan film containing lauric-arginate and nisin effectively reduced *S. aureus* and *L. monocytogenes* counts on the inoculated deli ham slices. All serogroups of *E. coli* including O₁₅₇: H₇, O₁₁₁, and O₂₆ were entirely eliminated when the raw meat slices were wrapped with the films supplemented with the aforesaid antimicrobial combination. Khare et al. (33) also postulated that chitosan in combination with cinnamon EO had a synergistic effect to prolong the shelf life of chicken meat nuggets. Only a few commercial mixtures of organic acids (Articoat DLP-02® and Sulac-01®) and extracts (Citrox®) used as meat coatings are available in the literature (36, 37). Besides, inorganic materials such as metals and metal oxides have been the focus of nanofood packaging research (17). Commercial films containing 1% nanoclay (Dellite® 67G) and an active-nano-iPP film containing 1% nano clay and 5% polyβ-pinene (Piccolyte® S115) have been used to prolong the shelf life of RTE meats (38). Among the AAs used in meat and meat products' packaging- reviewed by Woraprayote et al. (16) with an emphasis on bacteriocins. We will focus on essential oils (EOs) and nanotechnology meat packaging in the following section.

4. Essential oils

EOs extracted from spices and herbs are naturally occurring AAs which have been approved to show antibacterial, antiviral, antimycotic, and antitoxigenic characteristics in meat and meat-based products (13). EO compounds are categorized into two groups of the different biosynthetic source. The principal class contains terpenes (e.g. terpinene, p-cymene, limonene, and sabinene) and terpenoids (e.g. thymol, carvacrol, and linalool) whereas the other consists of aromatic phenylpropanoids (e.g. eugenol, and cinnamaldehyde) (39, 40). The EOs have the effect on microbial cells by various mechanisms, including but not limited to damaging the membrane phospholipid bilayer and proteins, disrupting enzyme systems, and inactivate or ruin genetic material (41-43). Pezeshk et al. (44) have reviewed the plant origin antimicrobial compounds for extended shelf-life of seafood. Unacceptable sensory deterioration restricts EOs' application in food packaging concerns. Higuera et al. (45) analyzed the microbiota of the packaged fresh chicken fillets—mesophiles, psychrophiles, *Pseudomonas* spp., *Enterobacteria*, LAB and yeasts, and fungi with chitosan/

cyclodextrin films supplemented with carvacrol, during storage. A general microbial suppression was observed but the abundant quantity of EO absorbed to or reacted with the fillet caused sensory rejection (45). Accordingly, utilization of hurdle technology in the application of EOs is recommended in order to make simultaneous progress in microbial safety and sensorial quality of meat products. The combination might take place with some other preservation technologies including MAP, high hydrostatic pressure (HHP) (46, 47), preservatives and low-dose irradiation (48-50). For instance, the combined influence of thymol, carvacrol, and temperature on the quality parameters of poultry patties (consisted of ostrich, chicken and turkey meats) packaged in air and MAP: 40% CO₂; 30% O₂; 30% N₂ was scrutinized. The effectiveness of EOs in lower concentrations was achieved at low temperatures under MAP conditions compared to the aerobic condition, regarding the better suppression of spoilage microorganisms and pathogens in the former circumstance. Furthermore; no off-odor was detected during the first 4 days of storage in both packaging atmospheres with amounts of EOs used in low temperature (51). The results of another study by Vardaka et al. (37) demonstrated that citrus extract plus chitosan could exhibit superior antimicrobial activity against *E. coli* O₁₅₇: H₇ and *Salmonella enterica* in vacuum-packed turkey meat stored at chill temperature, maintaining counts of these pathogens at low levels. Paparella et al. (52) also reported that chitosan combined with *Origanum vulgare* EO boosts its antimicrobial activity when applied for MAP packaging of fresh pork, with low sensory impact. Recent technologies e.g. encapsulation of EOs into nano-emulsions are also being used because of the aforementioned negative organoleptic effects. A list of recent research conducted in this area is presented in Table 1.

5. Nanoparticles

The European regulation (EU) Cosmetic Regulation (EC No 1223/2009) states that a nanomaterial is an “insoluble or bio-persistent and deliberately produced material with one or more external dimensions, or an internal structure, on a scale from 1 to 100 nm” (21, 53). When nanomaterials such as polymers, metals or composites are used they show different physical and chemical properties from their micro- or macro-scale counterparts made up of the same substance (54) being specifically effective, because of the large surface areas of the particles and enhanced surface reactivity of the nano-sized AAs (27). The ions or salts are of restricted usefulness for several reasons, including but not limited to the hinder effects of salts on the antimicrobial mechanism. These kinds of limitations can be conquered by the use of nanoparticles (NPs) (55). Comprehensive reviews on nanotechnology and its potential applications in the meat industry have been published by Baltic et al. (56) and Ramachandriah et al. (57). Nanocomposites are indeed composite materials in which the matrix material is reinforced by one or more separate NPs (58). The matrix material could be organic or inorganic based comprising polymers such as PA, PS, nylon, polyolefins etc.

(57) and bio-nanocomposites such as starch and cellulose derivatives, PLA, and polyhydroxybutyrate (PHB) (21). Polymer-clay nanocomposites provide nanosized dispersion which causes notable improvements in thermal stability and betterment of mechanical, barrier and physical properties of the polymer films. Nanoclays can also enhance the solubility of active substances in the polymer matrix which leads to the controlled release of active materials for longer periods (15, 53). The most likely looking nanofillers are layered silicate nanoclays e.g. montmorillonite (MMT) and kaolinite (21). Metal (Ag, Cu, Au, Pt) and metal oxide [titanium-dioxide (TiO₂), zinc oxide (ZnO), magnesium-oxide] NPs, and organically modified nanoclay [quaternary ammonium-modified MMT (e.g. Cloisite®15A, Cloisite®20A and Cloisite®30B)] are routinely used or tested antimicrobials in a wide variety of nano-packaging applications (7, 27). Alongside the type of NPs, shape, geometry size and surface charge, etc. are also key factors to define the antimicrobial action of NPs (57). Hence the packaging matrices are primarily characterized by means of microscopic and spectroscopic techniques. Images of the NPs interacted with the packaging materials can be obtained by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

The crystalline nature of the coatings and films and the analysis of particle sizes are also tested by X-ray diffraction (XRD). Besides, Fourier transforms infrared (FTIR) is used to reveal the functional groups and chemical changes after NPs incorporation (59-61). Mahdi et al. (62) carried out a study on the antimicrobial effect of Ag-NPs coated on PVC/PE laminate trays on microbial quality and shelf life of minced meat stored at the chill temperature (3±1°C). The shelf life of minced meat extent from 2 days to 7 days and microbial content was reduced. The packaged minced meat samples with lower thickness showed lower microbial loads. Fedotova et al. (63), also reported the strong bactericidal and fungicidal effect of Ag NPs in sausage casings made of cellulose and collagen derivatives. The antimicrobial mechanism of Ag-NPs has been associated with membrane destruction due to the derivation of free-radicals and also the accumulation of the NPs in the cytoplasmic membrane (Fig. 1). Kim et al. (55) discussed a feasible free-radical involvement near the Ag-NPs surface that induces antimicrobial effects, by electron spin resonance (ESR) measurements. However; in some cases, no antimicrobial activity of the Ag-NPs has been reported. Gallochio et al. (2016) explained that no relevant difference was observed overall in the tested bacteria levels (TVC, *Enterobacteriaceae* and *Pseudomonas* spp.) between meatballs preserved in plastic bags containing Ag-NPs or control bags (64). Metal oxide NPs have also been exploited in food packaging systems for their action against a spectrum of spoilage and disease-causing microorganisms (65). ZnO is somehow more appealing over Ag, being less toxic and cost efficient (53). Results of a study by Akbar and Anal (2014) in which ready to eat (RTE) poultry meats were wrapped by

ZnO-NPs loaded Ca-alginate films, indicated a high effect on inoculated target bacteria. *S. aureus* and *Salmonella* Typhimurium reduced 6-7 log-cycles within 10 days of incubation at 8±1°C, whereas no significant number of reductions observed in control samples inoculated with the same bacteria (66). The antibacterial action of ZnO-NPs has been related to the electrostatic interactions between the liberated ions and the microbial cell walls, the formation of reactive oxygen species (ROS) by the effect of light radiation, and subsequent destruction of cell wall architecture and cell lysis (Fig. 1) (67). The photocatalytic activity of metal oxide NPs has been substantiated due to the generation of extremely ROS under irradiation by a light source (7). Nanocomposite films reinforced with some chemically modified clays have also approved to have an antimicrobial function (21, 68). Moreover; nanocomposites with combined antimicrobials have been developed for meat packaging purposes as well. In a recent study Morsy and colleagues performed some initial experiments using plate overlay tests to assess the antimicrobial functionality of rosemary and oregano EOs and Ag and ZnO-NPs on target bacteria inclusive of *L. monocytogenes*, *S. aureus*, *E. coli* O₁₅₇:H₇ and *S. Typhimurium*. The results demonstrated the inhibition effects of the active compounds on some of the pathogens' survival in plate overlay tests. Pullulan films were also prepared in challenge studies to examine the antimicrobial role of active compounds against the selected pathogens in wrapped fresh or RTE meat and poultry products kept at 4°C for up to 3 weeks. Effective inhibition of the aforementioned bacteria survival was observed (69).

In another survey, Zimoch-Korzycka and Jarmoluk (70) prepared biologically active edible hydrosols based on hydroxypropyl methylcellulose, chitosan, lysozyme, and nanocolloidal Ag, which applied to the surface of meat samples. Antimicrobial action of different concentrations of chitosan, lysozyme and nanocolloidal Ag hydrosols against Gram+ bacteria: *Bacillus cereus* and *Micrococcus flavus* and Gram- bacteria: *E. coli* and *Pseudomonas fluorescens*, was assessed. Death of each tested bacteria was observed while covering meat samples by hydrosols containing chitosan and other bioactive compounds. Slight suppression of *M. flavus*, *E. coli*, and *P. fluorescens* growth has been detected when chitosan was omitted from the sol medium. Different organizations like USFDA detected a few migrations of nanomaterials from packaging into foodstuff but recognized them as safe and convenient because of extremely low migration (53, 71). In an aforementioned study (64) on the Ag migration from a commercially accessible food packaging loaded with Ag-NPs into chicken, the absence of Ag-NPs in chicken meatballs under the experimental circumstances was observed and this complies with the overall migration limit (a maximum amount of 0.010 mg kg⁻¹ for the migration of non-authorized substances through a functional barrier) as set by EU Commission Regulation No. 10/2011.

Table 1. Antimicrobial active packaging (AAP) systems performed by the incorporation of active substances into the packaging material applied in the meat industry (a summary since 2010).

Antimicrobial component			Packaging material	Packaged product	Technique	Process	Antimicrobial effect	References
Plant-derived	Nanoparticles	Miscellaneous						
-	Ag NPs	-	PVC/PE laminate trays	Minced beef	Layer-by-layer assembly	Inkjet-printed method	Low <i>E. coli</i> and <i>S. aureus</i> counts, Extended shelf life	(62)
Rosemary and oregano EOs	Ag and ZnO	-	Pullulan films	Meat and poultry	Embedding	Casting	Effective inhibition of the pathogens associated with vacuum packaged meat and poultry products	(69)
Oregano and thyme EOs	-	-	Soy protein edible films	Ground beef patties	Embedding	Casting	No significant effect on TVC, LAB, and <i>Staphylococcus</i> spp.; reductions in coliform and <i>Pseudomonas</i> spp. counts	(72)
-	-	Lysozyme and Na ₂ EDTA	Zein films	Ground beef patties	Embedding	Casting	Decreased TVC and total coliform counts	(34)
-	-	Sakacin	Pullulan films	Turkey breast RTE poultry meat	Embedding	Casting	Reduction of <i>L. monocytogenes</i>	(29)
-	ZnO NPs	-	Ca-alginate		Embedding	Casting	Reduction of inoculated <i>S. Typhimurium</i> and <i>S. aureus</i>	(66)
-	-	Lysozyme and lactoferrin	Paper containing CMC	Carpaccio	Non-covalent immobilization	Direct addition to the slurry	Reduced TAC	(73)
-	-	Chitosan lactate	LDPE	Sliced red meats	Embedding	Casting	No antimicrobial effect regarding TVC	(74)
Green tea	-	-	Chitosan film	Pork sausages	Embedding	Casting	Lower TAC and YMC	(25)
Clove	-	-	EVOH	Grass carp slice	Embedding	Extrusion	Extending shelf life	(10)
-	Ag substituted zeolite	Nisin, chitosan, potassium sorbate	LDPE-polyamide-active LDPE	Chicken drumsticks	Embedding	Extrusion	AMB, coliforms and YM inhibition	(75)
-	SiOx	-	Chitosan	Red drum fish	-	Casting	SiOx did not affect the antibacterial properties of the chitosan	(61)
Eugenol	-	-	LDPE	Chicken thighs	Embedding	Extrusion	Reducing the growth of <i>Pseudomonas fluorescens</i> and AMB	(76)
Pomegranate peel extract	-	-	Casein-based edible films	Ground beef	Embedding	Casting	Extended shelf life	(77)
Clove and cinnamon	-	-	Starch edible films	White shrimps	Embedding	Casting	Lower bacterial counts and shelf life extension	(23)
poly-β-pinene (PβP)	-	-	iPP-nanoclay-PβP	Salami	Covalent immobilization	Extrusion	Inhibition of total AMB, <i>Salmonella</i> ssp., <i>L. monocytogenes</i> and YM	(38)
Mustard glucosinolates	-	-	PPG	Bologna sausages	Embedding	Casting	Reduced <i>L. monocytogenes</i> viability and inhibited LAB growth	(78)
-	-	Chitosan-nanocellulose	Chitosan	Ground beef	Embedding	Casting	Decreased LAB population	(79)
-	-	Lauric arginate and nisin Z	Pullulan films	Turkey breast, deli ham, and raw meat	Embedding	Casting	Inhibiting the growth of <i>Salmonella</i> spp., <i>L. monocytogenes</i> , <i>S. aureus</i> , and <i>E. coli</i>	(35)

Table 1 continue...

Pomegranate peel extract	-	-	PE	Pork meat	Embedding	Extrusion	Lower TVC and extended shelf life	(80)
-	-	Active-PE	PET		Layer-by-layer assembly	-		
-	-	Ag-Zn zeolite Ag substituted TiO ₂	LDPE- active LDPE	Pork sirloin	Embedding	Extrusion	No extended shelf life	(81)
-	Ag-NPs	-	LDPE-NPs		Layer-by-layer assembly	Liquid flame spray		
Thymol, eugenol, and carvacrol	active compound loaded MMT and halloysite	-	LLDPE	Sucuk (fermented sausage)	Embedding	Extrusion blow molding	Suppression of total AMB, LAB, and YM; bacteriostatic effect on <i>E. coli</i> O ₁₅₇ :H ₇	(15)
Carvacrol	-	-	Chitosan/cyclodextrin	Chicken fillets	Embedding	Casting	Reduced growth of fungi and yeasts, mesophiles and <i>Enterobacteriaceae</i>	(45)
-	-	Nisin and EDTA	Nanocrystal /chitosan	Fresh pork loin meats	Non-covalent immobilization	Casting	Restricted the growth of psychrotrophs, mesophiles, and LAB and reduced <i>E. coli</i> and <i>L. monocytogenes</i>	(49)
Ethanol extract from coconut husk	Naturally occurring MMT (Cloisite Na ⁺)	-	Tilapia and squid skin gelatin films and nanocomposite	Mackerel meat powder	Embedding	Casting	Maintained quality and extended shelf life	(82)
-	Ag NPs	-	LDPE	Chicken breast fillets	Embedding	Extrusion	Delayed microbiological growth of bacteria; Lower counts of Gram ⁺ and Gram ⁻ bacteria	(83)
-	Ag NPs	-	PVC	Chicken breast fillets	Embedding	Casting	Reduced Gram ⁻ bacteria and extended shelf life	(84)
-	-	Pediocin	Cellulosic films	Bologna sausages	Embedding	Casting	Reduced <i>L. monocytogenes</i> growth	(67)
Thyme	-	-	Chitosan	Cooked cured ham	-	Casting	Lower AMB and LAB counts	(85)
-	-	Chitosan	Cellulose	Sausage	Embedding	Casting	Fewer TVC	(86)
Oregano EO	-	-	Chitosan	Petrovská klobása	Embedding	Casting	Better preservation properties	(87)
-	-	Lysozyme	Gelatin	Minced Pork	Layer-by-layer assembly	Casting	Lower TVC and YMC	(88)
Cinnamon oil	-	-	Chitosan	Chicken meat nuggets	Embedding	Casting	Extended shelf life	(33)
-	-	Ag-Sio ₂ , Ag-zeolite and Ag-Zn	PP	<i>In vitro</i>	Embedding	Extrusion	Better activity against Gram ⁻ than Gram ⁺	(89)
Ginger	-	-	Chitosan	Barracuda fish	Embedding	Casting	Effective against G ⁺ than Gram ⁻ and maximum antibacterial properties against <i>S. aureus</i> and <i>E. coli</i>	(90)
Carvacrol	-	-	PP/PP/ active EVOH	Salmon	Embedding	Extrusion	Not discussed	(91)
-	-	Lysozyme and lactoferrin	PET/gelatin	Salmon fillets	Layer-by-layer assembly	Casting		(92)

Table 1 continue...

Carvacrol	-	-	PP/PP/active EVOH		Embedding	Extrusion	Efficient in reducing <i>Shewanella putrefaciens</i> , <i>P. fluorescens</i> , psychotropic and mesophilic bacterial populations	
Clove EO	-	-	CMC–PVOH films	Ground chicken meat	Embedding	Casting	No inhibitory effect on <i>P. fluorescens</i> ; decreased TVC and complete elimination of <i>S. aureus</i> and <i>B. cereus</i>	(93)
-	-	ε-poly(lysine)	Silver carp surimi films	Fresh fish meats	Embedding	Casting	Suppression of the increase of TVC and prolonged shelf life	(94)
-	-	Chitosan	LDPE	Tilapia	Embedding	Extrusion	Inhibition of <i>E. coli</i>	(95)
-	-	ε-Poly(lysine)	EVOH	Surimi sticks	Embedding	Casting	Increased shelf life	(96)
Clove EO	-	-	Sunflower protein films	Sardine patties	Embedding	Casting	Reduced TVC, total mesophiles, H ₂ S-producers, luminescent colonies, <i>Pseudomonas</i> spp., LAB and <i>Enterobacteriaceae</i>	(97)
-	-	Chitosan	Chitosan films	Fillets of hake and sole	Embedding	Casting	Biocide effectiveness against total AMB, <i>Pseudomonas</i> , H ₂ S-producing bacteria, LAB, <i>Enterobacteriaceae</i> , and <i>L. monocytogenes</i>	(98)
Clove and pepper EOs	-	Chitosan	Grouper bone gelatin films	Fish steaks	Embedding	Casting	Inhibitory activities against <i>S. aureus</i> , <i>Aeromonas hydrophila</i> , and <i>L. monocytogenes</i>	(99)
-	-	Acetic acid and propionic acid	Chitosan	Dried anchovy	Embedding	Casting	Reduced aerobic plate count and YMC by acetic acid loaded films	(100)
Cinnamon EO	-	-	Agar and alginate bilayer	Chilled shrimps	Embedding	Casting	Decreased TVC, total AMB, H ₂ S-producers, and suppression of inoculated <i>L. monocytogenes</i>	(101)
-	-	Ethyl-N-α-dodecanoyl-L-arginate hydrochloride	EVOH	Chicken stock and RTE surimi sticks	Embedding	Casting	Exhibiting significant antibacterial effect against <i>L. monocytogenes</i> and <i>E. coli</i>	(102)
Thyme EO	-	-	Whey protein isolate	Trout	Embedding	Casting	Enhanced quality significantly	(103)
-	-	Chitosan	Chitosan–gelatin composite	Rainbow trout	Embedding	Casting	Dramatic reduction in TVC and Psychotropic bacteria	(104)
-	Tea polyphenols-encapsulated chitosan NPs	-	Sodium alginate	Tilapia fillets	Embedding	Casting	Significant inhibition of bacterial growth (TVC)	(105)
-	-	Lactoperoxidase system	Chitosan	Rainbow trout	Embedding	Casting	Decreased <i>Shewanella putrefaciens</i> , <i>P. fluorescens</i> , psychotropics, and mesophilic bacteria	(106)
Oregano EO	-	-	Silver carp skin gelatin-chitosan	Grass carp muscle	Embedding	Casting	Decreased <i>E. coli</i> , <i>S. aureus</i> , <i>Bacillus subtilis</i> , <i>Bacillus enteritidis</i> , and <i>Shigabacillus</i>	(107)
-	-	Chitosan, lauric arginate ester, sodium lactate, and sorbic acid	PLA	Pre-sliced turkey deli meat	Layer-by-layer assembly	Casting	Reduced the growth of <i>L. innocua</i> , <i>L. monocytogenes</i> , and <i>S. Typhimurium</i>	(24)
-	-	Silver-zinc crystals	PA/PE-pigment/active PA	<i>In vitro</i>	Embedding	Extrusion	Inhibiting the growth of <i>P. aeruginosa</i> , <i>P. chrysogenum</i> , <i>Lactobacillus acidophilus</i> , <i>L. monocytogene</i> , and <i>S. Typhimurium</i>	(108)

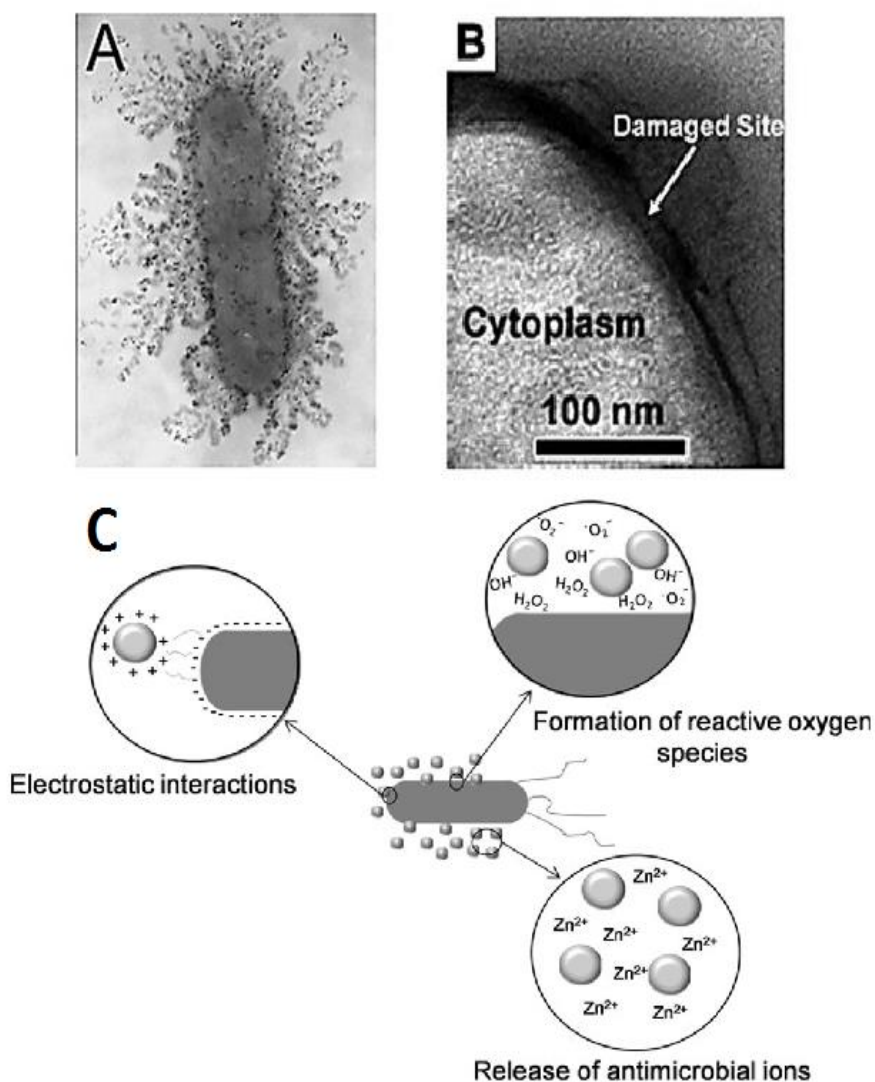


Fig. 1. Different mechanisms of antimicrobial action of Ag and ZnO-NPs. (A and B): the figure demonstrates adherence of Ag-NPs to and subsequent perforating of the membrane surface of *E. coli*. (54) (C): Mechanisms of ZnO-NPs bactericide (represented by gray spheres) (67).

Cushen et al. (109) in another study evaluated the migration of nano-Ag from plasticized PVC nanocomposites to chicken breast. The Ag content of the meat was measured by an inductively coupled plasma mass spectroscopy (ICPMS) and a migration range of 0.03–8.4 mg/kg was obtained by this method. According to a sensitivity analysis, Ag NPs' transfer from the nanocomposite to meat surface was influenced intuitively most by the filling percentage ($p < 0.01$), followed by storage duration ($p < 0.01$). The temperature did not seem to have any significant influence in this study. The amount of Ag present in meat samples was not substantially influenced by NP diameter ($p > 0.01$). Nanocomposite films reinforced with some chemically modified clays have also approved to have an antimicrobial function (21, 68). Moreover; nanocomposites with combined antimicrobials have been developed for meat packaging purposes as well. In a recent study Morsy and

colleagues performed some initial experiments using plate overlay tests to assess the antimicrobial functionality of rosemary and oregano EOs and Ag and ZnO-NPs on target bacteria inclusive of *L. monocytogenes*, *S. aureus*, *E. coli* O₁₅₇:H7 and *S. Typhimurium*. The results demonstrated the inhibition effects of the active compounds on some of the pathogens' survival in plate overlay tests. Pullulan films were also prepared in challenge studies to examine the antimicrobial role of active compounds against the selected pathogens in wrapped fresh or RTE meat and poultry products kept at 4°C for up to 3 weeks. Effective inhibition of the aforementioned bacteria survival was observed (69).

In another survey, Zimoch-Korzycka and Jarmoluk (70) prepared biologically active edible hydrosols based on hydroxypropyl methylcellulose, chitosan, lysozyme, and nanocolloidal Ag, which applied to the surface of meat

samples. Antimicrobial action of different concentrations of chitosan, lysozyme and nanocolloidal Ag hydrosols against Gram+ bacteria: *Bacillus cereus* and *Micrococcus flavus* and Gram- bacteria: *E. coli* and *Pseudomonas fluorescens*, was assessed. Death of each tested bacteria was observed while covering meat samples by hydrosols containing chitosan and other bioactive compounds. Slight suppression of *M. flavus*, *E. coli*, and *P. fluorescens* growth has been detected when chitosan was omitted from the sol medium. Different organizations like USFDA detected a few migrations of nanomaterials from packaging into foodstuff but recognized them as safe and convenient because of extremely low migration (53, 71).

In an aforementioned study (64) on the Ag migration from a commercially accessible food packaging loaded with Ag-NPs into chicken, the absence of Ag-NPs in chicken meatballs under the experimental circumstances was observed and this complies with the overall migration limit (a maximum amount of 0.010 mg kg⁻¹ for the migration of non-authorized substances through a functional barrier) as set by EU Commission Regulation No. 10/2011. Cushen et al. (109) in another study evaluated the migration of nano-Ag from plasticized PVC nanocomposites to chicken breast. The Ag content of the meat was measured by an inductively coupled plasma mass spectroscopy (ICPMS) and a migration range of

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6. Developing the AP systems for meat packaging.

It is well known that we have a film, in the case of housing the antimicrobial molecules in the whole packaging wall which is a self-standing separate layer; a coating in the case of coating an active layer onto a polymeric or paper substrate providing a bilayer or multilayer packaging; and an edible coating provided that the active adherent layer is directly coated onto the food (7). Therewith, to manifest antimicrobial function, AAs may be embedded within a packaging material or be kept in touch with the surface of the product (direct or indirect contact in the case of volatile compounds and direct contact in the case of non-volatile compounds (3, 110). All the described embodiments can be built in many different technologies, comprising: embedding for controlled release, immobilization, layer-by-layer displacement, and photo-grafting (Fig. 2).

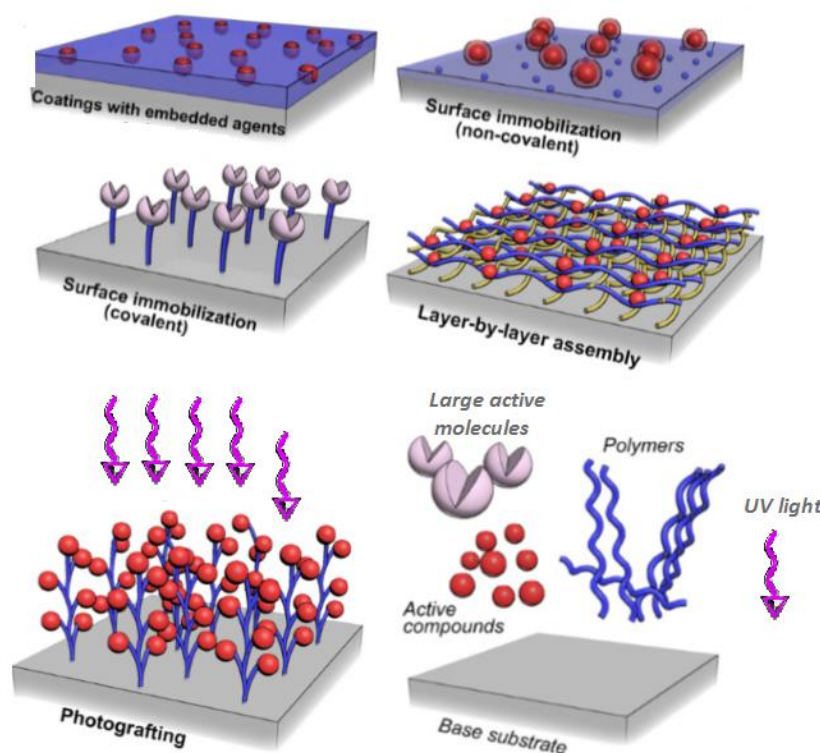


Fig. 2. Summary of coating technologies retrieved from an illustration by Bastarrachea et al. (3).

In migratory technologies, the active agent is intended to transfer to the packaged food (embedding, non-covalent immobilization, some layer-by-layer displacement

techniques) and to remain immovable in the packaging matrix (covalent immobilization, some layer-by-layer displacement techniques, photografting) in non-migratory systems since the

release of the AAs is not required or even forbidden (e.g. in the case of non-food-grade biocides) (3, 111). Two basic procedures are involved to build such active packages:

(i): Wet process (solution-casting) is fundamental on solubilization or dispersion of a polymeric matrix or blend containing active agents in a pertaining solvent medium to form a film-forming solution to be casted or sprayed onto an expedient substrate which could be either a packaging layer or foodstuff (7). In an aforementioned study by Siripatrawan and Noipha (25), for instance, green-tea-extract-embedded chitosan film was made ready by the casting of the film-forming solution on a ceramic plate and subsequent drying in ambient temperature. In another article by Lara-Lledó et al. (112) a film-forming solution was formed by dissolving a water-soluble polyvinyl PE glycol graft copolymer (PPG) in a water/ethanol mixture. After stirring and cooling, pure sinigrin, oriental and yellow mustard extracts were added to the solution singly. The antimicrobial solutions were cast in Petri dishes and then placed in an incubator at 60 °C for 1 h. The pouches containing inoculated (by in culture of *L. monocytogenes*) bologna sausages were vacuum-packed. The overall interpretation of the results was that sinigrin and mustard films reduced *L. monocytogenes* viability on packed bologna and also inhibited LAB growth on bologna stored up to 70 days.

(ii) The dry process often includes melt and extrusion techniques as well as hot-pressing or blow-molding to develop films (113). In the latter case, a rather high thermal stability of active compounds is mandatory because of the thermal severity of the process conditions (7). Barraza et al. (114) prepared active LDPE film containing eugenol extruded in a blown-extrusion apparatus. The results indicated that regardless of the high losses of eugenol during the extrusion process, a moderate antimicrobial effect on the growth of *P. fluorescens* and aerophilic mesophilic bacteria (AMB) was observed while contacting fresh chicken pieces under commercial storage circumstances. Nonetheless; extrusion can bring a highly efficient manufacturing method due to the equal distribution of AAs in the amorphous region of the packaging matrix (115). Besides, the rapid and continuous extrusion process offers the advantage of having a commercial potential for large-scale film production, over solution-casting (116). In a recent work by Yang et al. (117), active films were prepared by extrusion of EVOH with clove EO. The effects of EVOH/clove EO films on quality assessment of Grass Carp slice on the basis of sensory evaluation and biochemical indices during chilled storage (4±1 °C) were inspected over the period of 10 days. The interpretation of the results revealed that the 3% clove EO the loaded film could dramatically retain the fish freshness and prolong the shelf life to 7–8 days in comparison to the control group, which was only 4 days. In another study Soysal et al. (74) examined the effects of AAs (nisin, chitosan, Na-sorbate or Ag-substituted zeolite (AgZeo)) loaded LDPE (LDPE/PA/active LDPE-containing 2% AA) films manufactured using a blown film extrusion process, on the quality of vacuum-packed chicken drumsticks stored at 5 °C for 6 days compared with control bag (LDPE-

PA-LDPE). In all the sampling points total AMB, total coliforms and total YMC of the active packaged meats were lower than packaged samples in control bags. Hu et al. (118) also prepared pomegranate-peel-extract grafted polyethylene (PE) films by the extrusion blow molding process. Two double-layer active films including pomegranate-peel-extract-PE/PET and pomegranate-peel-extract-PE/PP were obtained with the active internal layer. The results demonstrated that the double-layer active films could prolong pork shelf life. By day 6, the TVC of active packaged pork samples was ~1 log-cycle lower than PE packaged samples. Among all the available literature we surveyed on the subject, in the majority of the studies performed, the antimicrobial substances were embedded into the packaging material matrix by means of casting or extrusion. Furthermore; surface immobilization has been reported as an approach to covalently or non-covalently bonded antimicrobial substances onto a functionalized solid support providing antimicrobial coatings for meat products. It is probably the least studied approach explored for AP applications. Barbiroli et al. (72) incorporated positively charged antimicrobial proteins lysozyme and lactoferrin into a paper matrix containing carboxymethyl cellulose (CMC), with non-covalent linkage in an approach for packaging of "carpaccio" (thin slices of veal fillet). The results showed that lysozyme/lactoferrin synergically performed the best on TAC in fillets after storage for 48 h in 4±1 °C, giving almost 1 log-cycle decrease compared with control. However, the lactoferrin-only paper was seemingly inefficacious against this certain meat microbiota.

Covalent-bonded immobilization needs the presence of functional groups on both the antimicrobials and the polymer site (119). The functional groups which require a molecular structure large enough to withstand efficacy after covalent immobilization onto a solid support, are limited to enzymes or further antimicrobial proteins (3, 11). In addition, immobilization usually requires the use of cross-linkers or "spacer" molecules (e.g. dextran, polyethylene glycol, ethylenediamine for food concerns) that bind the polymer surface to the bioactive agent (Fig. 2) (3). In a paper by Khan et al., nisin and EDTA were fixed on the surface of bio-nanocomposite films by using genipin (a cross-linking agent derived from the fruit *Genipa americana*) and γ -irradiation. Nisin was cross-linked onto the surface of the nanocrystal /chitosan nanocomposite films in order to protect its activity in meat packaging against glutathione which is a strong reducing agent in meat tissue. The films restricted the growth of psychrotrophs, mesophiles, and LAB in fresh pork loin meats and prolonged the shelf life of meat samples by more than 5 weeks. The films also decreased *E. coli* and *L. monocytogenes* populations in artificially contaminated meat samples (49). Ayhan et al. (38) studied the antimicrobial characteristics of commercial nanocomposites including iPP/organophilic clay and iPP/Poly- β -pinene/clay as alternatives to multilayer materials used in meat packaging. PP/PA/EVOH/PE a commercial multilayer alloy was used as a control in the case of in situ trial. Since covalently immobilized poly- β -pinene could not diffuse from the

packaging matrix, therefore poly- β -pinene containing materials were more efficacious in vacuum cling wrap due to direct contact with the surface of salami slices.

Photo-induced graft polymerization-allocated to surface modification of assorted polymers- is a kind of radical polymerization, where a synthetic reaction begins with generated radicals of monomers by exposure to UV light (120). This technique could be used to construct AAP either by direct assimilation of the AAs during photografting or by the following immobilization after grafting of a polymer chain with reactive functional chemicals (121). Shin et al. (122) developed a non-migratory antifungal LDPE polymer for use in food packaging applications. The natamycin-grafted LDPE films demonstrated antifungal activity against *Saccharomyces cerevisiae* and *Penicillium chrysogenum* on growth media and in food application. The film suppressed mycelium formation of *P. chrysogenum* up to 60%. In addition to the aforementioned procedures based on direct incorporation of AAs into packaging materials, it is possible to coat active substances on the surface of packaging substances as an individual layer which provides a high AA concentration in contact with the food surface. For this purpose, different coating treatments e.g. immersion or spraying with a coating/carrier solution could be applied. The AA is firstly dissolved in an appropriate solvent e.g. ethanol before applying to the substrate. Although layer-by-layer deposition technologies in meat packaging systems have mostly focused on ameliorating barrier properties, they can potentially be applied in AP as well (3). The facility of using a layer-by-layer approach in meat packaging concerns has been explored through a few studies (Table 1). The effectiveness of nisin-coated polymer films for controlling *S. Typhimurium* on fresh broiler skin has been investigated by Natrajan and Sheldon (123). In this study three films including PVC, LLDPE and nylon were coated with solutions containing 100 $\mu\text{g/ml}$ nisin and different concentrations of citric acid, EDTA, and tween 80. Broiler drumstick skins were placed in treated films and after inoculation with *S. Typhimurium* were stored at 4°C for 24 h. Authors found that the shelf life of refrigerated samples was increased and the populations of surviving *S. Typhimurium* organism on the surface of broiler skin and drumsticks were markedly reduced (123). Analogous results were obtained for hot dogs packaged in films coated with nisin by Franklin et al. (124). In a recent paper, authors ascertained the efficacy of films coated with a methylcellulose/hydroxypropyl methylcellulose-based solution composed of varying concentrations of nisin to reduce *L. monocytogenes* on the surface of vacuum-packed hot dogs. They claimed that films treated with the solution including 10000 and 7500 IU/ml nisin substantially reduced *L. monocytogenes* counts on the surface of packaged hot dogs throughout the 60 days of cold circumstance. In a more recent paper, Guo et al. (24) coated edible chitosan/acid solutions formulated with lauric-arginate ester, Na-lactate and sorbic acid alone or in combination on PLA packaging films. PLA films coated with chitosan, sodium-lactate, and sorbic acid significantly decreased the growth of *L. innocua* but were less effective

against *S. Typhimurium*. The combination of sodium-lactate and sorbic acid with lauric-arginate ester did not generate synergetic antimicrobial effect against *Listeria* or *Salmonella* on the meat surface.

Notwithstanding the foregoing, by reviewing the previous literature, we encountered a few cases where the expected antimicrobial effect of the active packaging was missed. Kuuliala et al. (125), for instance, determined the antimicrobial efficacy of Ag-containing LDPE packaging films cling-wrapped on fresh pork sirloin pieces. The shelf life of meat was not affected by any of the silver-containing packaging films, even though meat microbiota mostly consisted of bacteria that were suppressed or retarded in vitro by a nanoscale silver coating. The inefficiency of coextruded films was probably due to their structure. In another paper, Sun et al. (61) postulated that the preservation properties of the chitosan coatings on the red drum (*Sciaenops ocellatus*) were remarkable taking into account the freshness indicators of aquatic products, and that of in situ synthesis nano SiOx chitosan coatings were more superior. But in situ synthesized nano SiOx did not improve the antimicrobial performance, considering TVC, of the chitosan coatings. Park et al. (73) also observed that TVC Log values of meat surface did not decrease by wrapping with impregnated LDPE films by chitosan lactate, but a marked extension of red color shelf life was noted in chill temperature, through 10 days of storage.

7. Challenges and Perspectives

AAP can be a good platform for studying the related effects in order to provide an increased margin of safety and quality of raw meat and RTE meat products in modern society. The global marketing of active food packaging is expected to be around the US \$12,000,000,000.00 in 2017 (3). Many factors must be considered in the design of antimicrobial packaging systems. For example, in some investigations, it was shown that the film treatments were more effective against microorganisms in the culture media than in meat samples (24, 126) which proves the importance of checking the APS functionality in real food applications. As regard to incorporation processes utilized for active packaging preparations, the solution-casting method is an energy-consuming procedure which is only adequate for laboratory use; it cannot be applied in large-scale production of food packaging materials. Therefore, more efficient techniques are needed for commercial film production (104). The packaging matrix formulation (e.g. polymer concentration, selection of suitable solvent, plasticizers, dispersants, and emulsifiers) has to be optimized to engineer coating and film microstructure (76, 127). As an alternative, extrusion technology can industrialize the active packaging fabrication, but the loss of active compounds during processing due to direct heat exposure has been reported to be a major problem. Therefore, the films sustain a slight antimicrobial activity. Recently, encapsulation has been reported as a promising method to eliminate external destructive stimuli (128, 129). Appraisal of modification techniques such as photografting in order to

the efficiency of coatings and films has also recently become a research hot spot and critical issue. UV-initiated graft polymerization is one of the actively studied areas because of the fast reaction rate, low cost of processing, and proportionally simple process facilities (122). The application of such non-migratory active packaging films demonstrates a promising approach to maintain food quality with diminished additive concentration. The performance of the mathematical modeling of mass transfer of the antimicrobial substances in controlled release systems is required to in-depth understanding and subsequent optimization of the process parameters (130). Most of the mathematical models used for modeling the release kinetics of AAs and fit the data with convenient equations are fundamental on Fickian diffusion models (114, 117, 131). Appropriate boundary conditions and restrictive conditions of geometry should be used to properly model the process. Hereon, the unwanted migration of NPs from food packaging into food is a matter of concern because of their plausible ingestion, inhalation, or even transfer through dermal contact (57, 132). The migration of natural base nanomaterials such as microcrystalline cellulose or nanoclay to the food matrix has been observed (22). However, there is no particular legislation for even metal nanomaterials (132). Migration testing using standardized simulants must be done to quantify levels of migrants in packaged product systems. The biodegradability maintenance of biodegradable polymers produced using NPs is another matter of recent and current discussions (22).

Conclusion

By moving in the direction of novel theories and models that truly integrate what we are learning from researches, it seems to be distinctly possible that AP systems for muscle-based food products systems will become more commercially pragmatic and commonplace in the years to come. Cost issues and mechanical properties of the packaging system should also be taken to account in the final structural design.

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