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Antimicrobial food packaging: An overview

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Abstract
This study was focused on the antimicrobial packaging of foods which is one of the new active packaging concept developed as a response to the consumer's demands and market trends. At present food contamination is the major problem in the country which leads to spoilage and growth of pathogenic microorganisms. This can happen when food exposed to the environment during slaughtering, processing, packaging, and shipping. Although some traditional food preservation methods such as drying, heating, freezing, fermentation, and salting are used to extend shelf-life of food, but it is not consummate especially to inhibit the growth of pathogenic microorganisms that may endanger consumers' health. Antimicrobial packaging (AM) is a novel development that incorporates antimicrobial agent into polymer film to suppress the activities of targeted specific microorganisms. However, the uses of these packaging films containing antimicrobial agents are more efficient due to the mechanism of slow migration from the packaging material to the surface of food and they mainly follows the diffusion mass transfer mechanism due to its concentration gradient of the antimicrobial agent. This gradient mainly generated in the packaging system by the active package (higher AM conc.) and the contained food (none or low AM conc.). However, the antimicrobial packaging is still challenging technology and there only a few commercialized products found in the market.

Keywords: antimicrobial food packaging, food quality and safety

1. Introduction
Antimicrobial packaging is defined as a packaging system that interacts with the food product or the surrounding headspace either to kill microorganisms that might be present in the food product or to inhibit their growth [35, 2]. It can offer slow, continuous migration of the agent from the packaging material to the food or the headspace of the package so that an adequate concentration of the antimicrobial agent is maintained over the shelf life period of the product [30]. Consumption of healthy, natural foods has increased and become very popular during recent years. However, many of these food products are perishable and require protection from spoilage during their preparation, storage, and distribution. They may undergo faster deterioration as a result of physical, chemical and biological processes, including microbial contamination, which is mostly caused by bacteria, yeasts, and molds as a result of a low amount or lack of additives. The intrinsic microbial load of food is also closely related to the production of undesirable effects in foodstuffs, such as changes in odor, color, taste, texture, etc. These kinds of changes lead to a reduction in shelf life which decreases the safety of food and threatens the security of public health [19].
As a consequence, most of the food industry's main objectives are to produce safe, high-quality food products with a longer shelf life, and to develop innovative food preservation technologies capable of avoiding food borne diseases without compromising product quality and freshness, thus ensuring a safer global food supply [16]. For that purpose, antimicrobial packaging concept is mainly introduced it's the part of the active packaging. Basically, the purpose of this packaging is to provide safety assurance, shelf life extension, and quality maintenance of food, and inhabiting spoilage and suppressing food borne illness microbes that can potentially contaminate food products [2].
Traditionally, antimicrobial agents have been added directly to food products to extend their shelf life. However, this method is not always effective because of the possible interactions and chemical reactions produced by additives or ingredients during food processing. Moreover, the concentrations of the active agents may decrease owing to their diffusion into the food matrix and an excessive amount of additives is needed in order to achieve the antimicrobial effect, with the corresponding risk of modifying the organoleptic properties of the food [34, 2]. To avoid using an excessive amount of antimicrobial agent, one option is the use of sprays or dips to deliver the compound to the food surface. The use of packaging materials as carriers for various active substances, including antimicrobial compounds, is a promising option to avoid microbial contamination of foods, and preserve them from deterioration processes. In this sense, active films can ensure a sustained release of the antimicrobial to the food surface or headspace of the package during the storage of the product. The primary functions of a food package are to contain the product, protect it against physical and environmental damage and provide information [11]. However, in recent decades the concept of Antimicrobial Packaging has

advanced in science to satisfy the demand of customers for safe, high-quality food products.

2. Methodologies for antimicrobial packaging systems

There are basically two methodologies for producing antimicrobial packaging systems: a) The addition of an independent device, such as a sachet, pad or label containing the antimicrobial agent, which is separate from the food product and is included with the food in a conventional package, and b) The use of antimicrobial materials incorporated during the manufacture of the package, that is, the antimicrobial agent is incorporated in a layer of a multi-laminate structure; the active agent exerts its action by direct contact of the wall package with the food or via its release from the wall of the package to the food or headspace.

At present, the second methodology is the preferred alternative because the presence of an extraneous object inside the food package could be accidentally ingested or possibly be confused with a new sauce, sticker, toy or discount card misleading the function of the incorporated device. Any antimicrobial agent deliberately added to a packaging system to exert antimicrobial activity through its release into the food product must be nontoxic to human health, and this aspect is controlled by a number of regulatory agencies. There are some manufacturing techniques for incorporating antimicrobial agents into the walls of a packaging system. Then can basically be divided into two procedures such as

2.1 Addition of agents that are intended to be released.

2.2 Immobilization of agents in the package wall with no agent release.

2.1 Antimicrobial systems based on the release of active agents

In these systems, the antimicrobial compound inhibits microbial growth via its release from the polymeric material onto the food surface or into the surrounding headspace. The antimicrobial agent is mixed with the polymer either by dissolution in a common solvent system from which the film is formed or by melt blending of the agent within the barrel of an extrusion machine or equivalent device. In both cases the active compound is distributed in the polymer matrix, homogeneously when polymer and agent are chemically compatible, or heterogeneously, forming a multiphase matrix in which sites enriched with the agent can be distinguished. The steps involved in the release of the agent from the polymer matrix comprise diffusion to the surface of the matrix and partition in the interface between the matrix and the food or the headspace. The mechanism of action differs, depending on the volatility of the agents described in section (i) and (ii).

(i) Volatile compounds

They can be incorporated directly into the polymer solution if they are compatible, and an active film or structure can be obtained by evaporation of the solvent. Depending on the volatility of the agent, the drying process may result in considerable agent loss, limiting the efficiency of the incorporation procedure. Alternatively, the compound can be incorporated by melt blending in a mechanical thermal process such as extrusion. In this case, the agent is heated to polymer melting temperature, with potential losses by both degradation and evaporation during film cooling. Whichever procedure is used, the final polymer film acts as a reservoir for the antimicrobial agents that will be released to the headspace and from the headspace to the food product, primarily during storage. The volatility of the compound, and its solubility in the food system and the package walls determine the calculation of the concentration of the compound in the food, which should always be higher than the minimum inhibitory concentration (MIC), defined as the lowest concentration required to produce visible inhibition in the growth of microorganisms [17]. Controlled delivery of the volatile is desirable to reach the right concentration during the product's shelf life, and restriction of release before packaging is advisable in order to reduce exhaustion of activity during film storage. The use of volatile antimicrobial agents is especially recommended for irregularly-shaped solid foods that cannot be vacuum packaged, that is, where good contact between food and packaging material is not possible (Figure 1).

(ii) Non-volatile compounds

The non-volatility of the compound reduces the loss during processing, despite the possibility of degradation due to interaction with the polymer matrix or to the temperature required for film manufacture. The main difference with respect to the previous group of agents is that, because of their non-volatility, direct interface contact between the active film and the food is required for release and, therefore, for any antimicrobial effect on the packaged product. Again, for the activity to be efficient, the non-volatile compound concentration in the food portion where the action is required should be higher than the minimum inhibitory concentration (MIC) during the product's shelf life. Non-volatile compounds are transported directly from the packaging materials to the food surface and by diffusion to the food matrix, and their use is effective for semi-solid or liquid foods where there is excellent food/package contact and the antimicrobial is quickly distributed in the food, exerting its action on the whole product (Figure 2).Organic acids and their salts, enzymes, bacteriocins, natural extracts, etc. have been used as antimicrobial compounds in packaging applications. For example, natamycin, a strong antifungal compound, has been incorporated in various biopolymers [4], and nisin, a polypeptide produced by Lactococcus lactis, has been widely used in packaging film [14, 33].

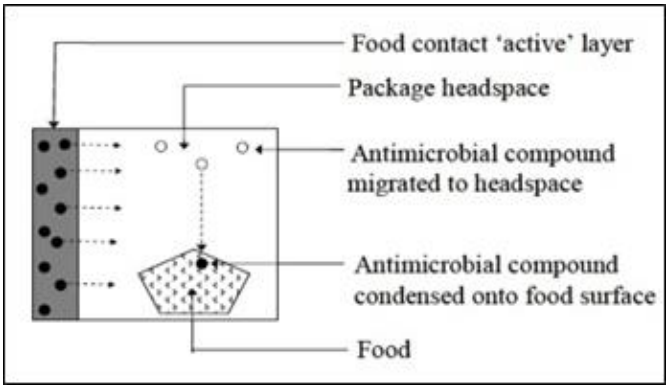

Fig 1: Mode of action of a volatile antimicrobial compound

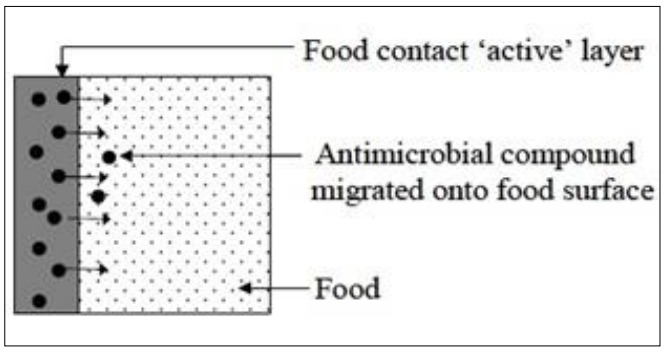

Fig 2: Mode of action of a non-volatile antimicrobial compound

2.2 Immobilization of antimicrobial agents on the surface of polymer film

Antimicrobial agents can be immobilized on the polymer surface of the package, exerting their antimicrobial activity by direct contact with the food product, or, more specifically, with the target microorganisms. This technology presents the advantage over the other technology that avoids the ingestion of the antimicrobial agent because they do not migrate to the food. Since the agents do not migrate, their activity is limited to the contact surface only (Figure 3). Immobilization of the antimicrobial has been used to create antimicrobial surfaces and it is convenient in the packaging of liquid foods were a good contact with the food is ensured, however, in solid or semi-solid foods, their activity is restricted to the area of contact between the packaging system and the food, and therefore their application is limited to vacuum packaging and/or the manufacture of slice separators. Peptide antimicrobials are among the agents commonly immobilized on film surfaces, especially bacteriocins such as nisin and enzymes such as lysozyme. However, the immobilization of these substances on conventional polyolefin films presents some difficulties. Their low surface energy, which results in poor printability and coating properties, is also responsible for their low bonding capacity with antimicrobial agents. To improve these functional characteristics, various surface treatment techniques have been attempted, that is wet chemical treatment with strong acids and bases; oxidation or ionization with ozone, corona and flame discharge [32].

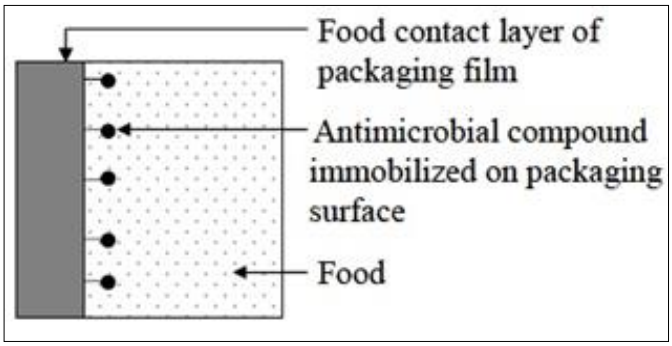

Fig 3: Immobilized antimicrobial packaging system

Table 1: Natural antimicrobials agents used into packaging of fresh and minimally processed fruits and vegetables.

Group	Type	Fruits or Vegetables	Target Microorganisms	Reference
Organic acid	Citric acid	Whole red organic apple and lettuce	*Escherichia coli* O157:H7 *Salmonella typhimurium*, *Listeria monocytogenes*	Park *et al.* (2011)
		Minimally processed Apple	Bacteria, yeast, and mold	Chauhan *et al* (2011)
	Lactic acid	Baby spinach	*Escherichia coli* O157:H7	Huang and Chen (2011)
		Lettuce	*Shigella species*	In *et al.* (2013)
Carbohydrate	Chitosan	Whole cantaloupe	*Salmonella*	Chen *et al.* (2012
		Minimally processed broccoli	Mesophilic bacteria, Psychrotrophic bacteria Yeast and molds Lactic acid bacteria Coliforms *Escherichia coli* O157:H7	Moreira *et al.* (2011)
		Apricot	*Burkholderia seminalis*	Lou *et al.* (2011)
		Pear	Fungi	Meng *et al.* (2010)
Plant extract	Bean broomrape	Table grapes Apricots Nectarines Oranges	Gray mold Brown rot Green mold	Gatto *et al.* (2011)
	Clove	Lettuce	*Salmonella typhimurium*, *Escherichia coli* O157:H7 *Listeria monocytogenes*	Kim *et al.* (2011)
	Apple Olive Hibiscus	Romaine Iceberg lettuce Spinach	*Salmonella enteric*	Moore *et al.* (2011)
Essential oil	Lemongrass	Minimally processed Pineapple	Yeast, mold, and total plate count	Azarakhsh *et al.*(2014)
	Oregano	Packed salad	*Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes*	Muriel-Galet *et al.* (2012)
	Grapefruit seed	Grape	Postharvest decay	Aloui *et al.* (2014)

3. Mechanism of antimicrobial packaging systems

Most of food packaging systems represent either a package/food system or a package/headspace/food system. A package/food system is a package in contact with a solid product, or a low viscosity/liquid food without headspace. Examples of food packages that can be included in this system are wrapped cheese, deli products, and aseptic meat packages. Diffusion between the packaging material and the food, and partitioning at the interface are main migration phenomena involved in such a system. A compound is incorporated into the packaging material can migrate into the food through diffusion, affected by partitioning, as shown in Figure 4. Package/headspace/food systems are represented by foods packed in flexible packages, cups, and cartons. Evaporation or equilibrated distribution of a substance among the headspace, packaging materials, and food is to be considered as part of

the main migration mechanism to estimate the interfacial distribution of an antimicrobial substance. Compared to a non-volatile substance, which can only migrate through the contact area between the package and the food, a volatile substance can migrate through the headspace and air gap between the package and the food [16], as shown in Figure 4.

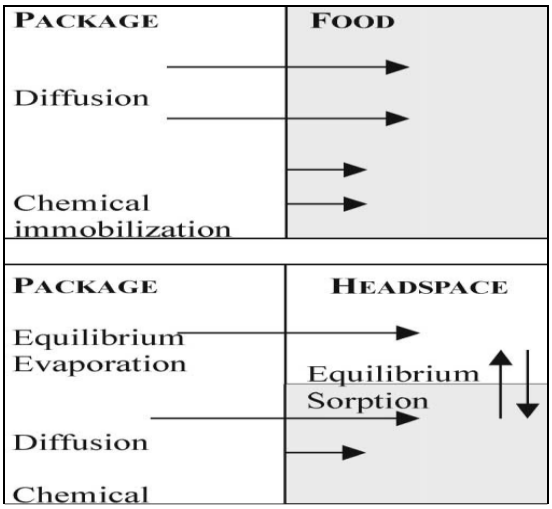

Fig 4: Food packaging systems and behavior of active substances

3.1 Controlled Release

For the design of an antimicrobial packaging system requires a balanced consideration of controlled release technology and microbial growth kinetics. When the mass transfer rate of an antimicrobial compound is faster than the growth rate of the target microorganism, the loaded antimicrobial compound will be diluted to less than the effective critical concentration (i.e. minimal inhibitory concentration, MIC) before the expected storage period is completed, and the packaging system will lose its antimicrobial activity because the packaged food has almost infinite volume compared to the volume of the packaging material and the amount of antimicrobial compound. Consequently, the microorganism will start to grow following depletion of the antimicrobial compound. On the contrary, when the migration rate is too slow to maintain the concentration above the MIC, the microorganism can grow instantly, before the antimicrobial compound is released. Therefore, the release rate of the antimicrobial compound from the packaging material to the food must be controlled specifically to match the mass transfer rate with the growth kinetics of the target microorganism. The solubility of the antimicrobial agents in foods is a critical factor of antimicrobial release. If the antimicrobial compound is highly soluble in food, the migration profile will follow an unconstrained free diffusion, while the very low solubility creates a dissolution-dependent monolithic system. For example, when highly soluble potassium sorbate was incorporated in packaging materials (e.g. plastic films or papers) and the antimicrobial packaging materials were used for semi-solid or high-moisture foods, such as paste, yogurt, fruit jelly, soft cheese and sliced ham, potassium sorbate dissolved in food immediately after packaging. Initially, potassium sorbate concentration increased very rapidly on the food surface and next to the surface concentration decreased slowly as potassium sorbate diffused into the food. Fast diffusion of the antimicrobial agents into food decreases their surface concentrations quite rapidly. Thus, the maintenance of required surface concentrations is highly dependent on the release rate from the packaging materials (i.e., the diffusivity of packaging materials) and the migration rate in the foods (i.e., the diffusivity of foods) [15].

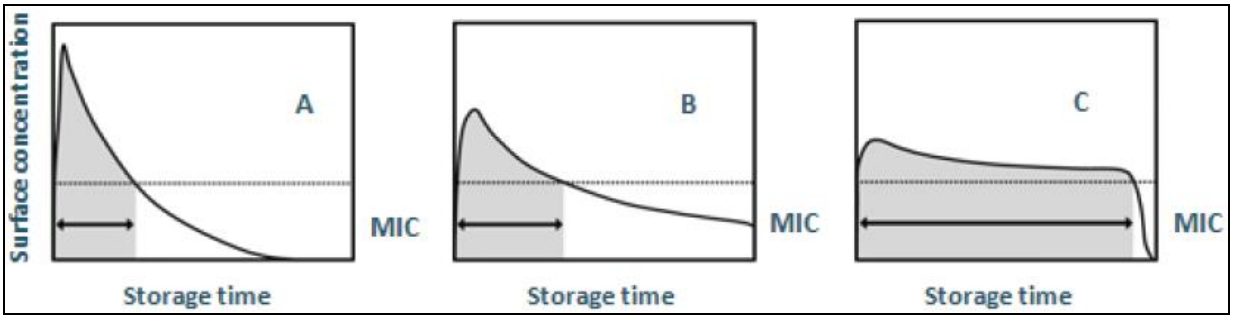

Fig 5: Release profiles of antimicrobial agents from different antimicrobial packaging films.

Release profiles of antimicrobial agents from three different antimicrobial packaging films are shown in Figure 5. System A is characterized by unconstrained free diffusion from packaging materials or a fast dissolution of antimicrobial tablets; system B shows slow diffusion of very low solubility agents from monolithic packaging materials; system C shows membrane (reservoir) system with constant flux of permeation, slow dissolution from antimicrobial powder/tablets or gaseous agent release from concentrated antimicrobial sachets/tablets with constant volatility in a closed packaging system. System C is the best, once the concentration of the antimicrobial is kept above the MIC (dashed line). Dashed lines and arrows indicate the MIC of a target microorganism, and the period of shelf life maintaining the surface concentration over the MIC, respectively [16].

4. Effectiveness of Antimicrobial Packaging

There are primarily two bases on which antimicrobial activity can be quantified: minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), which is presumably greater than the MIC. MIC is defined as “lowest concentration resulting in maintenance or reduction of inoculum's viability.” MBC is defined as “lowest concentration at which no growth is observed after

subculturing into a fresh broth." The MIC method is cited by most researchers, but some quote MBC as a measure of antibacterial performance [6].

4.1 Disc-Diffusion Method

The U.S. Food and Drug Administration (FDA) has approved this method as a standard for the National Committee for Clinical Laboratory Standards [36]. The disc-diffusion method is the most often-used technical method for antimicrobial screenings of essential oils (EO). As a paper disc soaked with the EO is placed upon the inoculated surface of an agar plate, and the zone of microbial inhibition is measured. Different parameters in this test could affect the results, such as the volume of EO on the paper discs, the thickness of the agar layer, and the solvent. For example, some reported solvents are ethanol, methanol, Tween-20, Tween-80, acetone in combination with Tween-80, polyethylene glycol, propylene glycol, n-hexane, and dimethyl sulfoxide, which could result in difficulties when comparing different studies [7-8].

4.2 Drop-Agar-Diffusion Method

The drop-agar-diffusion method was used for testing antimicrobial activity of extracts from the aerial parts of seven wild sages from Western Canada against bacteria, yeasts, and fungi, including Escherichia coli, Staphylococcus aureus, Candida albicans, and Aspergillus Niger [21].

4.3 Broth Microdilution Method

The broth microdilution method was used for the antimicrobial activity evaluation of aerial parts of fresh Plectranthus cylindraceus oil against Staphylococcus aureus and Bacillus subtilis [24]. This method showed lower MICs (% v/v) of different EOs extracted from the bay, clove, peppermint, and thyme against E. coli, S. aureus and C. albicans compared to agar-dilution assay. This method was used by [12, 31] to evaluate the antimicrobial activity of Acorus calamus against 17 species of bacteria, yeasts, and fungi.

4.4 Direct-Contact Technique in Agar

The direct-contact technique in agar has been used for screening the antimicrobial activity of carvacrol, the EO produced from Thymus ciliatus sp. Eu-ciliatus, against S. aureus and E. coli [6].

5. Use of Antimicrobial Packaging

Antimicrobial packaging can be considered an emerging technology that could have a significant impact on shelf life extension and food safety. Use of antimicrobial agents in food packaging can control the microbial population and target-specific microorganisms to provide higher safety and quality products. Many classes of antimicrobial compounds have been evaluated in film structures, synthetic polymers, and edible films [29]. To control food contamination and quality loss, edible coating or biodegradable packaging has been recently introduced in food processing. The packaging can serve as a carrier for antimicrobial and antioxidant compounds in order to keep a high concentration of preservatives on the food surfaces. Their presence could avoid moisture loss during storage, reduce the rate of rancidity causing lipid oxidation and brown coloration, reduce a load of spoilage and pathogen microorganism on the surface of foods, and also restrict the volatile flavor loss. The selection of the incorporated active agents is limited to edible compounds, and safety is also essential [29].

6. Conclusion

The study was concluded that growing packaging requirements and increasing focus on food safety, antimicrobial package has been widely used in food packaging. Yet, antimicrobial package is not simply a combination of antimicrobial agent and package; the two sides are interacted and mutually restricted. The most appropriate balancing point between the two sides is to be achieved with the help of material testing The use of antimicrobial packaging materials in food packaging can minimize the microbial contamination of food product surfaces during storage, transportation and handling. An interesting innovation could be the use of polymers which surfaces have been modified by electron irradiation or plasma treatment to generate antimicrobial activity without any transfer or migration of substances to the food.

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