Paper No.: 12
Paper Title: FOOD PACKAGING TECHNOLOGY
Module – 26: Antimicrobial food packaging

1 INTRODUCTION:

The demand for minimally processed, easily prepared and ready-to-eat 'fresh' food products, globalization of food trade and distribution from centralized processing pose important challenges for food safety and quality. Food-borne microbial epidemics are driving a search for innovative ways to inhibit microbial growth in the foods while maintaining quality, freshness and safety. One option is to use packaging to provide an increased margin of safety and quality. The next generation of food packaging may include materials with antimicrobial properties. These packaging technologies could play a role in extending shelf-life of foods and reduce the risk from pathogens. Antimicrobial polymers may be useful in other food contact applications as well. Antimicrobial food packaging acts to reduce, inhibit or retard the growth of microorganisms that may be present in the packed food or packaging material itself.

2 TYPES OF ANTIMICROBIAL PACKAGING:

Antimicrobial packaging can take several forms including:

1. Addition of packets/pads containing volatile antimicrobial agents into packages.
2. Incorporation of volatile and non-volatile antimicrobial agents directly in the polymers itself.
3. Coating antimicrobials on the surface of polymers.
4. Immobilization of antimicrobials onto polymers by various linkages.
5. Use of polymers that are having inherent antimicrobial properties.

2.1 Addition of packets/pads containing antimicrobial agents to packages:

The most successful commercial application of antimicrobial packaging has been sachets or packets that are enclosed loose or attached to the interior of a package. The forms predominated are oxygen and moisture absorbers & ethanol vapour generators. Oxygen and moisture absorbers are used primarily in bakery, pasta produce and meat packaging to prevent oxidation and water condensation. Although oxygen absorbers may not be intended to be antimicrobial, a reduction in oxygen inhibits the growth of aerobes, particularly molds. Moisture absorbers can reduce a_w, also indirectly affecting microbial growth. Ethanol vapour generators consist of ethanol encapsulated in carriers and enclosed in polymer packets. The ethanol permeates the selective barrier and is released in the headspace within the package. Since the amount of ethanol generated is relatively small and effective only in products with reduced water activity (aw<0.92), applications have been mainly to retard molds in bakery and dried fish products. Commercial examples include Ethicap, heat sealed packets containing microencapsulated ethanol in silicon dioxide powder, and Fretek, a paper wafer in which the centre layer is impregnated with ethanol in acetic acid and sandwiched between layers of polyolefin films. One of the drawbacks is the characteristic off flavour of ethanol.

2.2 Incorporation of antimicrobial agents directly into polymers:

Incorporation of bioactive agents including antimicrobials into polymers has been commercially applied in drug and pesticide delivery, household goods, textiles, surgical implants and other biomedical devices. Research on the incorporation of antimicrobials into packaging for food applications has increased more than 100% in the past 5 years. GRAS, non-GRAS and 'natural' antimicrobials have been incorporated into paper, thermoplastics and thermosets, and have been tested against a variety of microbes including *Listeria monocytogenes*, pathogenic *E. coli*, and spoilage organisms including molds. Of all the antimicrobials, silver substituted zeolites are the most widely used as polymer additives for food applications, especially in Japan. Sodium ions present in zeolites are substituted by silver ions, which are antimicrobial against a wide range of bacteria and molds. These substituted zeolites are incorporated into polymers like polyethylene, polypropylene, nylon and butadiene styrene at levels of 1-3%. Silver ions are taken up by microbial cells disrupting the cells' enzymatic activity. Commercial examples of silver substituted zeolites include Zeomic, Apacider, AgIon, Bactekiller and Novaron. Other compounds like antimicrobial enzymes such as lactoperoxidase and lactoferrin, antimicrobial peptides such as magainins, cecropins, defensins, natural phenols like hydroquinones and catechins, fatty acid esters, antioxidant phenolics, antibiotics and metals like copper and others may be useful.

The rationale for incorporating antimicrobials into the packaging is to prevent surface growth in foods were a large portion of spoilage and contamination occurs. For example, intact meat from healthy animals is essentially sterile and spoilage occurs primarily at the surface. This approach can reduce the addition of larger quantities of antimicrobials that are usually incorporated into the bulk of the food. The gradual release of an antimicrobial from a packaging film to the food surface may have an advantage over dipping and spraying. In the latter processes, antimicrobial activity may be rapidly lost due to inactivation of the antimicrobials by food components or dilution below active concentration due to migration into the bulk food matrix. Emulsifiers and fatty acids are known to interact with nisin reducing the bacteriocin's activity.

Antimicrobials may be incorporated into polymers in the melt or by solvent compounding. Thermal polymer processing methods such as extrusion and injection moulding may be used with thermally stable antimicrobials. Silver substituted zeolites, for example, can withstand very high temperatures (up to 800 °C) and therefore have been incorporated as a thin co-extruded layer with other polymers. For heat-sensitive antimicrobials like enzymes and volatile compounds, solvent compounding may be a more suitable method for their incorporation into polymers.

Antimicrobial packaging materials must contact the surface of the food if they are non-volatile, so the antimicrobial agents can diffuse to the surface, therefore, surface characteristics and diffusion kinetics become important. Antimicrobial release from the polymer has to be maintained at a minimum rate so that the surface concentration is above a critical inhibitory concentration. To achieve appropriate controlled release to the food surface, the use of multilayer films (control layer/matrix layer/barrier layer) has been proposed. The inner layer controls the rate of diffusion of the active substance while the matrix layer contains the active substance and the barrier layer prevents migration of the agent towards the outside of a package.

2. 3 Coating or adsorbing antimicrobials to polymer surfaces:

Early developments in antimicrobial packaging incorporated fungicides into waxes to coat fruits and vegetables and shrink films coated with quaternary ammonium salts to wrap potatoes. Other early developments included coating wax paper and cellulose casings with sorbic acid for wrapping sausages and cheeses.

Antimicrobials that cannot tolerate the temperatures used in polymer processing are often coated onto the material after forming or are added to cast films. Cast edible films, for example, have been used as carriers for antimicrobials and applied as coatings onto packaging materials and/or foods. Examples include nisin/methylcellulose coatings for polyethylene films and nisin/zein coatings for poultry. Proteins have an increased capacity for adsorption due to their amphiphilic structure. Nisin adsorbed onto silanized silica surfaces inhibited the growth of *L. monocytogenes*. Other examples include: adsorption of nisin on PE, EVA, PP, polyamide, PET, acrylics and PVC, pediocin containing milk-based powders adsorbed onto cellulose casings and barrier bags and nisin/EDTA/citric solutions coated onto PVC, nylon and LLDPE films.

Manipulating the solvents and/or polymer structures can enhance antimicrobial adsorption. Polyethylene- co-methacrylic acid films treated with sodium hydroxide and swollen with acetone showed an increased absorption and diffusion of benzoic and sorbic acids compared to non-treated films. These NaOH-treated films also had the highest inhibitory effect on molds. The explanation is that the higher polarity of NaOH-treated films enhanced the absorption of the antimicrobials. Binders such as polyamide resins have also been used to increase compatibility between polyolefins surfaces and bacteriocins. Glucose oxidase has been coated onto moisture proof fabric sheets by using polyvinyl alcohol, starch and casein as adhesives.

2.4 Immobilization of antimicrobials by ionic or covalent linkages to polymers:

This type of immobilization requires the presence of functional groups on both the antimicrobial and the polymer. Examples of antimicrobials with functional groups are peptides, enzymes, polyamines and organic acids. Examples of polymers used for food packaging that have functional groups are EVA, EMA, Ionomer, Nylon, PVDC etc. In addition to functional antimicrobials and polymer supports, immobilization may require the use of 'spacer' molecules that link the polymer surface to the bioactive agent. These spacers allow sufficient freedom of motion so the active portion of the agent can contact microorganisms on the food surface. Spacers that could potentially be used for food antimicrobial packaging include dextrans, polyethylene glycol, ethylenediamine and polyethyleneimine, due their low toxicity and common use in foods.

Ionic bonding of antimicrobials onto polymers allows slow release into the food. However, diffusion to the product is less of a concern when the antimicrobial is covalently bonded to the polymer unless conditions within the product promote reactions such as hydrolysis. This may occur for example, during the heating of a high acid food. Lysozyme and chitinase, both active against Gram positive bacteria, have been covalently immobilized. But, the activity was too low to be practical for packaging commercial applications. Glucose oxidase catalyzes the reaction between glucose and oxygen yielding the antimicrobial hydrogen peroxide. This enzyme has been covalently bound onto insoluble supports that could be compatible with packaging materials. Beta-galactosidase and glucose oxidase have been co-immobilized with the objective of producing hydrogen peroxide to activate lactoperoxidase in milk. Other antimicrobial enzymes that could potentially be covalently immobilized for packaging applications include lactoferrin, sulfhydril oxidase and bile-salt stimulated lipase. A major challenge, is the incorporation of substrates into the system as well as managing undesirable products from the reactions. For example, glucose oxidase needs glucose as a substrate, which could be provided by the food or added externally. Lactoperoxidase requires hydrogen peroxide and thiocyanate, commonly present in milk but not in many other foods. In both systems, hydrogen peroxide may raise toxicological concerns if amounts exceed the regulatory levels.

2.5 Use of inherently antimicrobial polymers:

Some polymers are inherently antimicrobial and have been used in films and coatings. Cationic polymers such as chitosan and poly-L-lysine promote cell adhesion since charged amines interact with negative charges on the cell membrane, causing leakage of intracellular constituents. Chitosan has been used as a coating and appears to protect fresh vegetables and fruits from fungal degradation. Although the antimicrobial effect is attributed to antifungal properties of chitosan, it may be that the chitosan acts as a barrier between the nutrients contained in the produce and microorganisms. In addition, chitosan-based antimicrobial films have been used to carry organic acids and spices. Calcium alginate films reduced the growth of the natural flora and coliform inoculum on beef, possibly due to the presence of calcium chloride. Bactericidal acrylic polymers made by co-polymerizing acrylic protonated amine co-monomer have been proposed as packaging materials for increased fruit and vegetable shelf life.

Physical modification of polymers has been investigated as means to render surfaces antimicrobial. For example, the antimicrobial potential of polyamide films treated with UV irradiation has been reported. Antimicrobial activity was presumably the result of an increase in amine concentration on the film's surface. Positively charged amine groups present in polymer surfaces may enhance cell adhesion but not necessarily death. It is possible that in the tests mentioned, simple adsorption occurred, masking the lack of antimicrobial activity of the laminated polymer surface. In a study on UV-treated nylon films it is seen that the surface amino groups were bactericidal, but that bacterial cells were adsorbed to the surface and diminished the effectiveness of the amine groups. In many cases, these studies are conducted in buffer. Addition of nutrients could potentially prevent cell membrane damage and bacterial recovery and/or inhibit the adhesion of the cells to the surface due to the interaction of salts and other cations with the surfaces.

3. Mode of antimicrobial function in the package

Antimicrobial functions which are achieved by adding antimicrobial agents in the packaging system or using antimicrobial polymeric materials show generally three types of mode; (i) release, (ii) absorption and (iii) immobilisation. Release type allows the migration of antimicrobial agents into foods or headspace inside packages, and inhibits the growth of microorganisms. The antimicrobial agents can be either a solute or a gas. Absorption mode of antimicrobial system removes essential factors of microbial growth from the food systems and inhibits the growth of microorganisms. For example, the oxygen-absorbing system can prevent the growth of moulds inside packages. Immobilisation systems do not release antimicrobial agents but suppresses the growth of microorganisms at the contact surface. Immobilisation systems are less effective in the case of solid foods compared to the liquid foods because there is less possibility for contact between the antimicrobial package and the whole solid food products.

4. Applications for food preservation

For both migrating and non-migrating antimicrobial materials, intensive contact between the food product and packaging material is necessary and therefore potential food applications include especially vacuum or skin-packaged products, e.g. vacuum-packaged meat, fish, poultry or cheese. Until now only few antimicrobial methods have found applications as a food-packaging material. But, as research in the area continues, more systems are showing promise for commercial applications.

4.1 Meat products

Studies have focused on establishing methods for coating LDPE film with methyl cellulose as a carrier for nisin. These films have significantly reduced the presence of *Listeria monocytogenes* in solutions and in vacuum packaged hot dogs. Use of chitosan to inhibit *L. monocytogenes* and chlorine dioxide sachets for the reduction of *Salmonella* on modified atmosphere-packaged fresh chicken breasts was also observed. Suitability of the antimicrobial films with incorporated lactic acid or sodium lactate, for packaging of raw chicken meat was studied. The chicken meat packaged in lactic acid/sodium lactate releasing films was storable at 4 °C for 7 days and retardation of microbial growth was by more than 2 log cycles.

4.2 Fruits, Vegetables and Cereals

Oranges when coated with an emulsion containing an essential oil (including basil essential oil) or a volatile compound (*Citrus reticulata* Blanco) had a longer shelf life than uncoated ones. Oranges treated with geraniol were rendered almost completely free from blue mold decay. Other treatments, in a decreasing order of effectiveness, were found to be mentha and basil essential oils. Basil oils can cause total inhibition of fungal (*A. flavus*) development on maize kernels. The optimal dosage for protection of maize was 5% (v/v) with hexane as the solvent. In addition, no phytotoxic effect on germination and corn growth was detected with this oil.

Fresh salad can be preserved by delivering essential oils to the product in the washing solution of the vegetables, or a food packaging film containing these oils can also be used.

4.3 Dairy products

Effect of polyethylene films coated by commercial PVC lacquer with addition of nisin (5% w/w) and/or natamycin (10% w/w) on the growth of target bacteria, yeasts and moulds on the surface of soft cheese was observed. The cheese in contact with nisin/natamycin treated film was stored at temperature 4°C and 23°C for 23 days, released antimicrobial agents caused retardation of microorganism growth by more than 1 log cycle on the surface of cheese Blatacke zlato. Nisin activated chitosan package when used for milk, the antimicrobial package retards the microbial growth and lowers the maximum growth levels in raw milk, pasteurized milk and UHT milk.

5. Conclusion

Antimicrobial packaging is a rapidly emerging technology. The need to package foods in a versatile manner for transportation and storage, along with the increasing consumer demand for fresh, convenient and safe food shows a bright future for antimicrobial packaging. However, more information is required on the chemical, microbiological and physiological effects of such systems on the packaged food, especially on the issues of nutritional quality and human safety. So far, research on antimicrobial packaging has focused primarily on the development of various methods and model systems. Little attention has been paid on its preservation efficiency in actual foods. Research is needed to identify the types of food that can benefit most from antimicrobial packaging materials. In order to have safe food, amendments to regulations might require toxicological and other testing of compounds prior to approval for use of antimicrobial packaging.

Antimicrobial packaging can play an important role in reducing the risk of pathogen contamination, as well as extending the shelf-life of foods; it should never substitute for good quality raw materials, properly processed foods and good manufacturing practices. It should be considered as a hurdle technology that in addition with other non-thermal processes such as pulsed light, high pressure and irradiation could reduce the risk of pathogen contamination and extend the shelf-life of perishable food products. Participation and collaboration of research institutions, industry and government regulatory agencies will be key on the success of antimicrobial packaging technologies for food applications.

Reference:

Ahvenainen R, 2003, *Novel food packaging Techniques*, CRC Press LLC, Boca Raton FL 33431, USA