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Antimicrobial Packaging for Extending the Shelf Life of Bread—A Review

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Antimicrobial packaging is an important form of active packaging that can release antimicrobial substances for enhancing the quality and safety of food during extended storage. It is in response to consumers demand for preservative-free food as well as more natural, disposable, biodegradable, and recyclable food-packaging materials. The potential of a combination of allyl isothiocyanate and potassium sorbate incorporated into polymers in providing the needed natural antimicrobial protection for bread products is discussed. The role of double extrusion process as a means for obtaining a homogeneous mix of the sorbate into the polymer (polyethylene or ethylenevinyalcohol), is highlighted.

Keywords Active packaging, antimicrobial packaging, bread, allyl isothiocyanate, sorbate, fungi, shelf life

INTRODUCTION

Active packaging material is defined as a type of material that extends the shelf-life or improves the safety or sensory properties of food while maintaining its quality (Vermeiren et al., 1999; Quintavalla and Vicini, 2002). Active packaging systems interacts with the food products or the head space between the package and the food system (Labuza and Breene, 1988; Rooney, 1995; Brody et al., 2001). The main aim of an active packaging system is to change the condition of packaged food in order to extend the shelf life of the food (Ahvenainen, 2003). Active packaging materials that can release active compounds for enhancing the quality and safety of an extensive range of foods during extended storage are particularly important. Antimicrobial packaging is a form of active packaging.

Plants in their wild state possess defense mechanisms against bacterial and fungal invasions, hence natural antimicrobials have been identified in spices and herbs and many studies have reported the preservation action of spices and their essential oils (Lopez-Malo et al., 2007). Antimicrobials derived from plants include eugenol from cloves, thymol from thyme and oregano, carvacrol from oregano, vanillin from vanilla, allicin from garlic, cinnamic aldehyde from cinnamon, and allyl isothiocyanate from mustard (Alzamora et al., 2003; Lopez-Malo et al., 2005). These antimicrobial agents as well as organic acids and their salts may be incorporated into packaging materials (Han, 2000). They reduce the growth rate of microorganisms by direct contact of the package with the surface of the foods and liquids. Antimicrobial packaging could be sanitizing or self-sterilizing which reduces chances of recontamination of processed foods. The concept of self-sterilizing foods is especially applicable to liquids (Appendini and Hotchkiss, 2002). Antimicrobial packaging systems may have a continuous active antimicrobial function that acts as an extra hurdle to kill undesirable microorganisms or inhibit their growth during storage and distribution (Han, 2003).

Fungi are the most common spoilage microorganisms of bakery products. Besides the unsightly appearance of visible growth, fungi are also responsible for off flavor formation and the possible production of mycotoxins and allergenic compounds which may be present even before growth is visible (Nielsen and Rios, 2000). Mould spoilage of bakery products during storage is a serious economic problem. The water activity of bakery products (0.75–90 a_w) makes them susceptible to xerophilic moulds-causing spoilage (Marin et al., 2002). These moulds are destroyed during baking and contamination arises from mould spores derived from the atmosphere or from the surfaces during the cooling, finishing, and wrapping procedures (Seiler, 1988).

The traditional method of preserving food from the effect of microbial growth include thermal processing, drying, freezing, refrigeration, irradiation, modified atmosphere packaging (MAP) and addition of antimicrobial agents or salts (Kerry et al., 2006). Freezing, refrigeration, and addition of

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antimicrobial agent or salts are commonly used for preserving bread. Refrigeration is not very effective as the loaf still undergoes staling under refrigeration temperature and hence losses its freshness. Freezing though effective requires that the loaf be allowed to thaw resulting in an increase in energy consumption. Addition of antimicrobial agent seems to be a waste of resources since microbial contamination of bread products occurs primarily at the surface, due to postprocessing handling. Consumers demand for preservative-free food as well as more natural, disposable, biodegradable, and recyclable food-packaging materials make this method unacceptable (Cha and Chinnan, 2004; Lopez-Rubio et al., 2004). Besides, synthetic additives have to be declared on the package, which will negate the current consumer desire for clean labeling. Antimicrobial packaging is, therefore, a promising form of active packaging for bread products. Our objectives were to review active packaging systems used in food products and to propose systems that may have potential for extending the shelf life of bread.

PRINCIPLES OF ACTIVE PACKAGING FOR FOOD PRODUCTS

Active packaging involves the incorporation of agents in the packaging that can either interact directly with the packaged food or with the atmosphere inside the package (Gutiérrez et al., 2009). Active packages, apart from providing an inert barrier to external conditions perform some desired role in food preservation (Hutton, 2003). It is a good alternative to both the use of preservatives and MAP. MAP has been used to extend the shelf-life of bakery products such as wheat bread, rye bread, hot-dog bread, and soy bread (Rodriguez et al., 2000; Nielsen and Rios, 2000; Fernández et al., 2006). Large numbers of pores in the bread matrix tend to trap oxygen, thereby making it difficult to reduce the oxygen content within the package (Galić et al., 2009). A solution is to use oxygen absorbers inside the package (Latou et al., 2010). However, MAP is expensive and in cereal products the growth of moulds and yeasts is not successfully inhibited, with dramatic decrease in sensory characteristics (Gutiérrez et al., 2011). The different forms of active packaging are outlined in Figure 1.

Active packaging provides extra functions including scavenging/absorbing (oxygen, CO_2, ethylene, moisture, flavors, odors, and UV light); emitting/releasing (CO_2, ethanol, SO_2, adsorption of flavors, pesticides, antimicrobial activity, and antioxidant release); removing properties (lactose and cholesterol); temperature (insulating materials, self-heating, self-cooling, microwave susceptors, modifiers, and temperature sensitive) and microbial and quality control [UV and surface-treated] (Kerry et al., 2006). Currently, viable and promising active packaging systems are the scavengers/absorbers specifically oxygen scavenging systems. They can (1) prevent rancidity by absorbing oxygen (Rooney, 1981) and (2) prevent growth of moulds and aerobic bacteria (Vermeiren et al., 1999) as well as control insect infestation in cereal products during storage, eliminating the need for chemical means of control (Nakamura and Hoshino, 1983; Vermeiren et al., 1999). These oxygen scavenging systems are developed in sachet form or polymer additives. The existing oxygen scavenging techniques uses one or more of the following concepts, namely, iron powder oxidation, ascorbic acid oxidation, photosensitive dye oxidation, enzymatic oxidation, for example, glucose oxidase and alcohol oxidase, unsaturated fatty acids such as oleic or linolenic acid, immobilized yeast on a solid material (Vermeiren et al., 1999). A potential risk with the iron oxidation systems in bread products is accidental ingestion of a large amount of iron, despite the label "Do not Eat." Furthermore, oxygen-free atmosphere can favor the growth of anaerobic pathogens such as *Clostridium botulinum* (Labuza, 1987). Oxygen scavengers can be used alone or in combination with MAP. In practice, most of the atmospheric oxygen in the pack is removed by MAP and then the residual oxygen within the package is removed by the oxygen scavenger system (Vermeiren et al., 1999). Enzyme systems are very sensitive to changes in pH, a_w, salt, temperature, and other factors in addition to requiring water (Vermeiren et al., 1999). The emitters and the release systems of active packaging are suitable for bread products especially the antimicrobial release/emitters.

FUNCTIONALITY OF ANTIMICROBIALS FOR ACTIVE PACKAGING OF FOOD PRODUCTS

Antimicrobials in food are used to enhance the quality and safety of food by reducing surface contamination of processed food; they, however, must not be used as substitute for good sanitation practices (Brody et al., 2001; Cooksey, 2005). The active packaging technologies designed to protect food products from deterioration and from microbial growth can involve the use of synthetic or natural antimicrobial agents (Kuorwel et al., 2011).

Synthetic Antimicrobials

Common synthetic antimicrobial chemicals are organic acids and their salts, sulfites, nitrites, antibiotics, and alcohols. Table 1 details the groups of substances used as antimicrobials in food systems. Antimicrobials reduce the maximum population of microorganism by reducing the growth rate and thus extending the lag phase of microorganism or inactivating the microorganisms (Quintavalla and Vicini, 2002). Antimicrobials may be incorporated into the packaging materials for slow release onto the food surface or may be used in vapor form (Wilson, 2007). Various types of antimicrobial agents have been experimented with to find suitability in antimicrobial packaging including organic acids, fungicides,

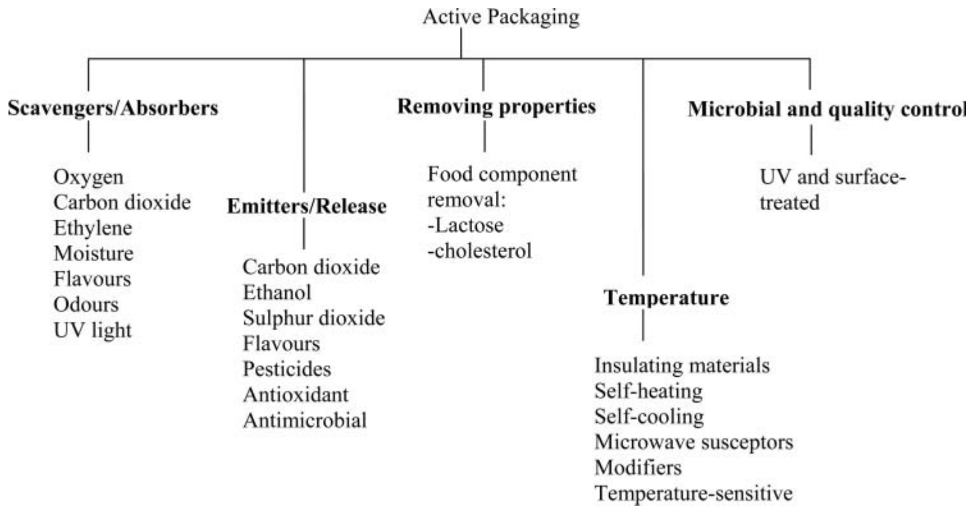

Figure 1 Active packaging systems (adapted from Kerry et al., 2006).

bacteriocins, proteins, enzymes, inorganic gases, and metal substitute's zeolite (Ming et al., 1997; Scannell et al., 2000).

Organic and inorganic materials or their salts may be used as antimicrobial agents in food packaging (Cahan et al., 2003). Organic acids such as sodium benzoate and potassium sorbate are the most widely used preservatives in the world and has obtained GRAS status. Most large commercial bakeries use them as a single ingredient or in a combination of the two to extend the shelf life of the bread. However, sorbates inhibit yeast fermentation, causing them to mainly be used as a spray-application after baking. Sorbates have a neutral effect on flavor and aroma (Gerdes, 2004). Many researchers reported that potassium sorbate was the most effective preservative to be used in bakery products (Marin et al., 2002; Guynot 2002). Bakery products are normally kept at room temperature (25°C), favoring the growth of xerophiles (Guynot et al., 2005). The most important factors controlling the growth of fungi on foods are temperature, pH, and water activity. Guynot et al. (2005) reported that potassium sorbate (0.3%, pH 4.5) was the most effective at preventing fungal spoilage of bakery products regardless of water activity. Potassium sorbate at 0.3% was found to be effective at inhibiting some fungal isolates from bakery products (Marin et al., 2002). Sorbic acid, which is soluble in fats and oils, and potassium sorbate, which is soluble in water, prove effective against a broad range of bacteria and moulds. Sorbic acid has an inhibitory role as a membrane active compound; this makes its activity less linked to the pH of the medium as benzoic acid and propionic acid (Stratford and Anslow, 1998). The current accepted theory of weak acid preservation is that inhibition takes place via internal pH depression by directly inhibiting glycolysis enzymes (Lück and Jager, 2000). The undissociated acid molecule, which is lipophilic and, therefore, ready to permeate the membrane on entering the cytoplasm, tends to

Table 1 Examples of typical antimicrobial agents used in food packaging

Class of antimicrobial agents	Examples
Organic acids	Propionic, benzoic, sorbic, acetic, lactic, malic, succinic, tartaric
Mineral acids	Phosphoric acid
Inorganics	Sulfites, sulfur dioxide
Parabens	Methyl, propylparaben
Antibiotics	Natamycin
Enzymes	Lactoperoxidase, lysozyme, lactoferrin
Metals	Silver, copper
Chelating agents	Ethylene diamine tetra acetate, purophosphate, citrates
Bacteriocins	Nisin, pediocins
Fungicides	Benomyl, Imazalil
Essential oils	Eugenol, thymol, salicylaldehyde, cinnamic acid
Proteins	Conalbumin, cathepsin
Phenolic antioxidants	Butylatedhydroxyanisole, Butylatedhydroxytoluene 2-terbutylhydroquinone
Isothiocyanates	Allyl isothiocyanate, hypothiocyante
Fatty acids and esters	Monolaurin
Others	Reuterin (3-hydroxypropionaldhyde), hydrogen peroxide, ozone, sulfur dioxide

Adapted from Hotchkiss (1997).

dissociate due to the internal pH being close to 7, delivering hydrogen ions and anions (Gould, 1996).

With the increase in consumer awareness for food safety, there is a general concern for use of chemical agents in food products (Azaz et al., 2005). Hence, many researches had been focusing on the use of natural antimicrobial compounds and their effectiveness in inhibiting microorganisms in food

Natural Antimicrobial Compounds

Many varieties of spice oleoresins and essential oils extracted from spices are used extensively in the food industry as flavoring agents. They were originally used for their flavor attributes, but recent scientific evidence supports their preservative ability. Natural antimicrobial compounds reported to inhibit microbial growth in solution, on culture media or on a variety of foods include organic acids, bacteriocins (nisin and lacticin), grape seed extracts, spice extracts (thymol, p-cymene, and cinnamaldehyde), lemon seed extracts, enzyme [peroxidase and lysozyme] (Han, 2000; Cutter, 2002; Conte et al., 2007); pepper (Careaga et al., 2003), orange extact (Fernández-Lopez et al., 2005), honey (Mundo et al., 2004), and propolis extract (Choi et al., 2006). Table 2 lists functions of some typical natural and synthetic antimicrobial agents that are used in food packaging.

Essential oils are extracts from plant materials some with known antibacterial, antifungal, antioxidant, and anticarcinogenic properties (Bagamboula et al., 2004). Antimicrobial activity of essential oils is associated with phenolic compounds (Davidson and Naidu, 2000). Hulin et al., (1998) reported that purified compounds such as carvacrol, eugenol, linalool, and thymol derived from essential oils inhibited a variety of microorganisms. Thymol inhibited the widest spectrum followed by carvacrol (Dorman and Deans, 2000). Several extracts from grape seed, spices (cinnamon, garlic, clove, and oregano) or their essential oils, and lemon seed have been reported to be able to inhibit bacterial growth in solution, on culture media or on a variety of foods (Cutter 2002; Conte et al., 2007).

Cinnamon oil and clove oil in combination at 2% in potato dextrose agar have been reported to inhibit the growth of seven mycotoxigenic moulds (*Aspergillus flavus*, *A. parasiticus*, *A. ochraceus*, *Penicillium* sp. M46, *P. roqueforti*, *P. patulum*, and *P. citrinum*) for various times up to 21 days (Azzouz and Bullerman, 1982). Manso et al. (2013) reported that the release of cinnamon essential oil from the polymer to the microorganisms occurs in the same manner irrespective of the plastic films (PET, PP, and PE/ ethylenevinylalcohol (EVOH)). Hence, the active compounds are integrated in the coating formulation, and the interaction with the polymer surface (absorption, degradation) is negligible (Manso et al., 2013). Nevertheless, plastic films require much less cinnamon essential oil than active paper to inhibit the mould as a result of the differences in the coating or porosity of the materials. However, both substrates indicate excellent long-term effectiveness and stability. Essential oils from aromatic plants including thyme and basil have been reported to inhibit fungal development on maize kernels (Montes-Belmont and Convajal, 1998).

Mustard essential oil has been used for inhibition of fungal growth on bread (Nielsen and Rios, 2000). Mustard seed essential oil contains an active compound called allyl isothiocyanate (AITC). AITC exists in a precursor form and upon cell destruction it is enzymatically hydrolyzed to release the active form (Nielsen and Rios, 2000). The precursor of AITC

Table 2 Antimicrobial agents and their function

Antimicrobial agent	Functions
Silver ions	Silver salts function on direct contact, but they migrate slowly and react preferentially with organics. One product is already in use called FresherLonger (NSTI, 2006).
Ethyl Alcohol	Absorbed onto silica or zeolite is emitted by evaporation and is effective but leaves a secondary odor.
Chlorine dioxide	This is a gas that can permeate through the package product. It is broadly effective against most microorganisms but has secondary effects such as darkening of meat and bleaching of green vegetables.
Nisin	It is most effective against Gram positive and lactic acid bacteria. It incorporates itself in the cytoplasmic membrane of target cells and works best in acidic environments (Cooksey, 2005).
Imazalil	Imazalil is a systemic imidazole fungicide used to control a wide range of fungi on fruit, vegetables, and ornamentals, including powdery mildew on cucumber and black spot on roses. Imazalil is also used as a seed dressing and for postharvest treatment of citrus, banana, and other fruit to control storage decay. It was chemically coupled to plastic films to delay the growth of moulds (Weng and Hotchkiss, 1992).
Organic acids	Acetic, benzoic, lactic, tartaric, and propionic acids are used as preservatives (Cha and Chinnan, 2004).
Spice based essential oils, e.g., ally isothiocyanate	It is and active component in wasabi, mustard, and horseradish it is an effective broad spectrum antimicrobial and antimycotic. It has a strong adverse secondary odor.
Metal oxides	Magnesium oxide and zinc oxide at nanoscale levels are being explored as antimicrobial materials for food packaging (Garland, 2004).
Ethylene absorbers	Ethylene is a natural plant hormone produced by ripening of fruit and vegetables, it accelerates produce respiration thus causing fruit to ripen and rot at a faster rate. The most common ethylene removal agent is potassium permanganate, which oxidizes ethylene to acetate and ethanol (Lopez-Rubio et al., 2004).

is found in common plants such as mustard, broccoli, horseradish, cabbage, cauliflower, kale, and turnips and its vapor form is more effective as an antimicrobial than in its liquid form (Lin et al., 2000; Kim et al., 2002, Shin et al., 2010). AITC from natural sources is used as a food preservative in Japan, and as a GRAS flavoring agent in the United States (Kim et al., 2002, Seo et al., 2012). The antifungal mechanisms of isothiocyanates have not yet been explained (Mari et al., 2008). A series of hypothesis have however been explained. One hypothesis which proves to be the most realistic is that a nonspecific and irreversible interaction of isothiocyanates with the sulfidryl groups, disulfide bonds, and amino groups of proteins and amino acid residues may take place (Kojima and Oawa, 1971; Banks et al., 1986). Lin et al. (2000) showed that if bacteria cells are fumigated with AITC it causes cell leakage resulting in cell death. A lipophilic compound 2-phenylethyl-isothiocyanate can react with enzymes present in the plasma membrane thus inhibiting cell growth and causing cell death (Troncoso-Rojas et al., 2005). From the density of commercial AITC, it was calculated that samples treated with 0.5 and 1.0 mL of AITC would have contained 0.49 and 0.97 mg of AITC. If the packaging headspace volume is 500 mL, the theoretical AITC concentration would be 1900 μg/mL when 1 mL was used (Nadarajah et al., 2005). At 3.5 μg/mL gas phase AITC is fungicidal on all fungi (Nielsen and Rios, 2000). The antimicrobial effects of AITC have been used to prepare a preservative system called WasaOuro [Table 3] (Wasaouro, 2010). This antibacterial agent is the product of a research and development project that employed the latest technology and expertise to harness the antimicrobial activity of allyl mustard oil for the purpose of foodstuff preservation. Wasaouro is currently used for product quality maintenance and sanitary management of food products as it prevents food spoilage and decreases the risk of food poisoning by preventing bacterial growth. It has also been shown to suppress bacteria and mould growth and extend the shelf life of bread for 7 days at room temperature due to its powerful action against the outbreak of moulds (Wasaouro, 2010).

The use of naturally derived antimicrobials is often preferred due to less complex regulation processes and lower perceived risk to the consumer when compared to synthetic antimicrobial agents (Baratta et al., 1998; Nicholson, 1998). Food products require higher levels of essential oils and their compounds for effective inhibition of microbial growth than culture media. This is due to many reasons such as (1) interactions between phenolic compounds and the food matrix (Nychas and Tassou, 2000) and (2) essential oils partitioning its hydrophobic component into the fat content of the food may prevent them from coming into contact with the microbial cells growing in the hydrophilic regions of the food (Gill et al., 2002). Hence, applications that do not require the direct incorporation of the essential oils in the food may be more effective. Although test conditions, microorganisms, and source of the antimicrobial compound, affect the results, some spices or essential oils always act very effectively in inhibiting growth (Nielsen and Rios, 2000).

Table 3 Applications of antimicrobial food packaging

Antimicrobial agent	Packaging material	Food
Organic acid		
Potassium sorbate	LDPE	Cheese
Calcium sorbate	CMC/paper	Bread
Propionic acid	Chitosan	Water
Acetic acid	Chitosan	Water
Benzoic acid	PE-co-MA	Culture media
Sodium benzoate	MC/chitosan	Culture media
Sorbic acid anhydride	PE	Culture media
Benzoic acid anhydride	PE	Fish fillet
Fungicide/bacteriocins		
Benomyl	Ionomer	Culture media
Imazalil	LDPE	Cheese
Nisin (peptide)	Silicon coating SPI	Culture media
Peptide/protein/enzyme		
Lysozyme	PVOH, nylon	Culture media
Glucose oxidase	Alginate	Fish
Alcohol/thiol		
Ethanol	Silica gel sachet	Culture media
Oxygen absorber/antioxidant		
Reduced iron complex	Sachet (Ageless)	Bread
BHT	HDPE	Cereal
Gas		
CO_2	Calcium hydroxide sachet	Coffee
SO_2	Sodium metabisulfite	Grape
Other		
UV irradiation	Nylons	Culture media
Silver zeolite	LDPE	Culture media
Grapefruit seed extract	LDPE	Soya sprouts

LDPE, low-density polyethylene; MC, methyl cellulose; CMC, carboxyl MC; PE, polyethylene; MA, met hacrylic acid; SPI, soya protein isolate; PVOH, polyvinyl alcohol; HDPE, high-density PE; BHT, butylated hydroxyl toluene.
Source: Adapted from Han (2000).

ANTIMICROBIAL PACKAGING SYSTEMS

These are polymeric materials in which a certain amount of additives with pronounced antimicrobial properties are embedded into the rigid or flexible plastic packaging container with the aim of extending the shelf life of foodstuffs (Appendini and Hotchkiss, 2002; Suppakul et al., 2003). Thus, packaging can be used to provide an increased margin of safety and quality of food products by incorporating antimicrobial properties into the packaging materials. Various forms of antimicrobial systems are detailed in Figure 2. Food packaging materials can obtain antimicrobial activity by coating, incorporating, immobilizing, or surface modifying antimicrobial substances, radiation, or by gas emission onto packaging materials (Suppakul et al., 2003). Various radiation techniques are used such as radioactive materials, laser-excited materials, UV-exposed film, or far-infrared-emitting ceramic powders (Han, 2000). Gas emission controls mould growth, examples are sulfite flushing in the fruit industry.

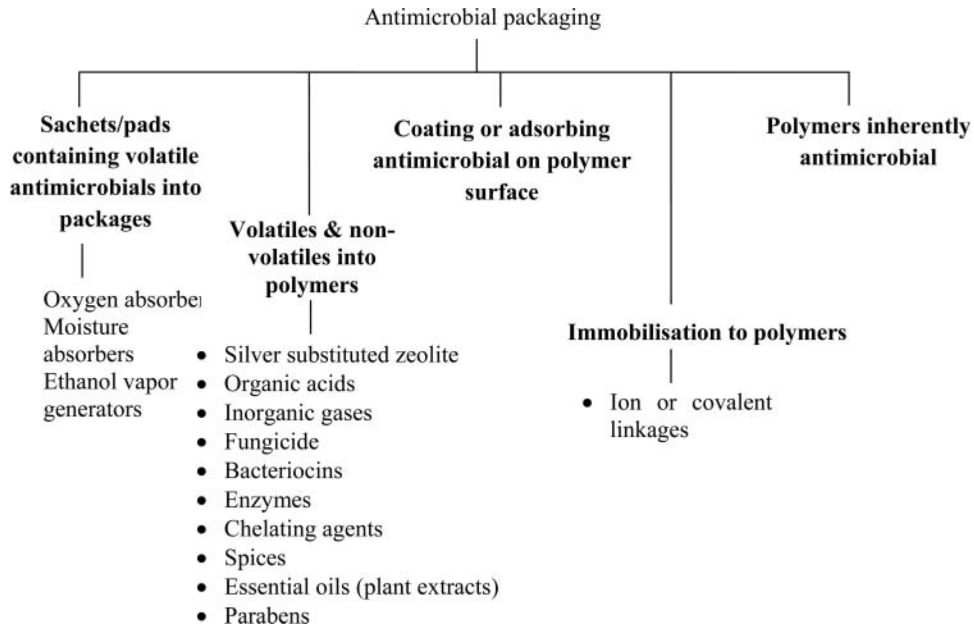

Figure 2 Antimicrobial packaging systems (adapted from Appendini and Hotchkiss, 2002).

Sachet systems are used inside a package, for example, ethanol-vapor-generating sachet which can inhibit mould growth on bakery products (Smith et al., 1987). Oxygen scavenging systems removes oxygen and prevents the growth of aerobic microorganisms, especially mould and it also reduces oxidation of food (Smith et al., 1986). Hence, antimicrobial packaging can be classified into two types (1) those that contain an antimicrobial agent which migrates to the surface of the food (migrating system) and (2) those which are effective against surface growth of microorganism without migration (nonmigrating system) (Kerry et al., 2006). In a migrating system, the antimicrobial is released from the packaging film into the package headspace and onto the food surface (packaging/headspace/food system) (Figure 3a). This system is most useful when direct contact between the packaging film and food product is not required for efficient antimicrobial activity (Cooksey, 2000). In this system, the antimicrobial has to be volatile so as to enter the headspace in the gaseous phase and then make contact with the food as shown in Figure 3a. Such release systems include chlorine dioxide, sulfur dioxide, carbon dioxide, and allyisothiocyanate. Volatile antimicrobials penetrate the food matrix without the polymer making direct contact with the food. In this packaging system, the food may be packaged in flexible packages, bottles, cans, cups, and cartons (Han, 2000). In nonmigrating system, the antimicrobial is immobilized within the packaging material (Brody et al., 2001). This system (packaging/food system) is applicable where direct contact between the food and the material can be achieved or is required for effective antimicrobial activity. The system describes food that is in direct contact with the package, making it possible for the nonvolatile antimicrobials to migrate directly onto the food product as shown in Figure 3b. Examples of this system include individually wrapped cheese, deli products and aseptic brick packages. Hence, diffusion between the packaging material and the food and partitioning at the interface are the main migration phenomena involved in this system (Brody et al., 2001). To achieve appropriate controlled release to the food surface, Floros et al. (2000) proposed the use of multilayer films (control layer/matrix layer/barrier layer). The inner layer (control layer) regulates the rate of diffusion of the active substance. The matrix layer contains the active substance and the barrier layer prevents migration of the substance towards the outside of a package. For example, cheese wrappers containing antimicrobials or a liquid in direct contact with the container without a headspace. The distinction between the two systems depends on the specific antimicrobial used and its interactions with the packaging and food matrix (Cooksey, 2000). Both synthetic and natural antimicrobials can be used in either packaging systems. Some recent antimicrobial systems are outlined on Table 4. One of them Ethicap is an ethanol emitter, which has been reported to reduce the growth of mould for 13 days thereby extending the shelf life of bakery products (Franke et al., 2010). The growth of mould was inhibited for 13 days with Ethicap. The prebaked bun has to absorb the ethanol to be effective in growth suppression of the *Bacillus* spp. and moulds. It absorbs most of the ethanol from the package headspace, and the ethanol content of the products is ~0.8 weight% after 21 days. This largely exceeds the overall migration limit of 60 mg kg^{-1} (0.006 weight%). Absorption of ethanol by the food product and the possibility of residual flavor is a major drawback as the concentration may cause regulatory problems (AgION, 2004).

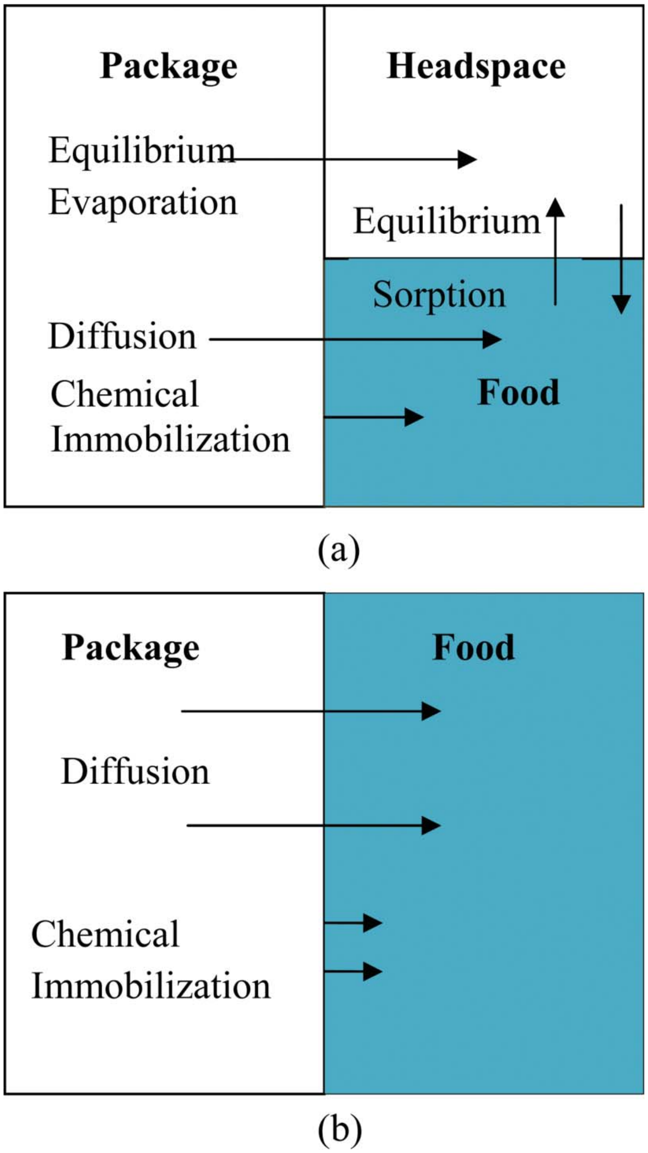

Figure 3 Diffusion of antimicrobial agent in a (a) package/headspace/food system and (b) package/food system (Brody et al., 2001).

*FreshPax Oxygen Absorbing Packets and Strips i*s a patented oxygen absorber developed by Multisorb Technologies to protect packaged foods and other products against spoilage (Table 4). Produced in sachet form using food grade ingredients, FreshPax irreversibly absorbs oxygen inside sealed sachet to less than 0.01% and maintains this level (Multisorb, 2010). Even under harsh testing conditions, the use of FreshPax resulted in a decrease in mould spoilage of commercial bread and cheese, with a lengthy extension of shelf life. The studies showed FreshPax also reduced the formation of *n*-hexanal and other volatile compounds in high-fat snack foods susceptible to oxidative rancidity. *FreshPax s*ignificantly improves keeping qualities of polyunsaturated fats and oils, helps retain fresh-roasted flavor of coffee and nuts, prevents oxidation of spice oleoresins, in spices themselves and in seasoned foods, prevents oxidation of vitamins A, C, and E and extends life of pharmaceuticals, inhibits mould in natural cheeses and other fermented dairy products, delays nonenzymatic browning of fruits and some vegetables and inhibits oxidation and condensation of red pigment of most berries and sauces. FreshPax can be used effectively with many kinds of packaging materials including EVOH and polyvinylidene chloride. FreshPax sachets are designed to be used in combination with current packaging methods including gas flushing and vacuum packaging.

AgION's (Table 4) customized antimicrobial solutions incorporate silver ions in a zeolite carrier. The silver ions interact with other positive ions (often sodium) from the moisture in the environment, effecting a release of silver ions on demand (AgION, 2010). The patented multi-faceted zeolite crystal carrier provides a three-dimensional release mechanism that provides efficient release of silver ions independent of particle orientation in the substrate. Silver ions contained in Zeolite crystals are randomly oriented and distributed through the surface of a fiber, polymer, or coating. In conditions that support microbial growth, positive ions, in ambient moisture, exchange with silver ions at reversible bonding sites on the zeolite. The exchanged silver ions are now available to reduce or eliminate microbial growth. Silver ions attack multiple targets in the microbe to prevent it from growing. This tri-modal action fights cell growth in three ways, it prevents respiration by inhibiting transport functions in the cell wall; inhibits cell division (reproduction) and disrupts cell metabolism. Depending on the microorganism, Agion's antimicrobial technology has been shown to initially reduce microbial populate ions within minutes to hours while maintaining optimal performance for years.

Microsphere (Table 4) powder releases chlorine dioxide gas above 50% humidity below that it is stable (Microsphere, 2010). It destroys pathogenic bacteria in the package environment. It is useful in control of decay and waste caused by fungi for the protection of minimally processed fresh vegetables and fruits. It is capable of destroying fungi, example *Aspergillus*, *Botrytis*, *Penicillium* through sustained and controlled release of chlorine dioxide upon exposure to humidity >80% and light and it leaves no residue on the food surface. It has high activity against a broad spectrum of microorganisms including vegetative cells and spores. Microsphere powder can be applied as a powder, in tablet form, in sachets, incorporated into adsorbent pads, incorporated into a LDPE master batch to extrude plastic film or bags with antimicrobial properties. Incorporated into coatings and adhesives incorporated into packaging forms, for example, paper and cardboard. The FDA has accepted that Microsphere powder as a GRAS (generally recognized as safe) compound. Triclosan (Table 4) is a white powdered solid organic compound with a slight aromatic/phenolic odor. It is a chlorinated aromatic compound that has functional groups of both ethers and phenols. Phenols have antibacterial properties. Triclosan has slight solubility in water, but is completely soluble in ethanol, methanol, diethyl ether, and strong basic

Table 4 Summary of some recent antimicrobial packaging developments

Antimicrobial agent	Trade name	Producer	Packaging type
Silver zeolite	AgIon	AgIon Technologies	Paper, milk containers
	Novaron	Toagosei Co. Ltd	Plastic
Triclosan	Microban	Microban Products	Deli-wrap, reheatable containers
Allyl isothiocyanate	WasaOuro	Lintec Corporation	Labels, Sheets, Sachets
		Dry Company Ltd	Sachets
Chlorine dioxide	Microsphere	Bernard Technology Inc.	Bags, coatings, labels
Carbon dioxide	Freshpax	Multisorb Technologies	Sachets
	Verifrais	SARL Codimer	Sachets (France)
Ethanol vapor	Ethicap, Negamold, Fretek	Freund	Sachets
	Oitech	Nippon Kayaku	Sachets (Japan)
Glucose oxidase	Bioka	Bioka Ltd	Sachets (Finland)

Adapted from Han (2000) and Appendini and Hotchkiss (2002).

solutions such as 1 M sodium hydroxide. Triclosan can be made from 2, 4-dichlorophenol (Triclosan, 2010). It has been in use since 1972, and it is present in soaps (0.10–1.00%), deodorants, toothpastes, shaving creams, mouth washes, and cleaning supplies, and is infused in an increasing number of consumer products, such as kitchen utensils, toys, bedding, socks, and trash bags. Triclosan is effective in reducing and controlling bacterial contamination on the hands and on treated products.

DESIGNING ANTIMICROBIAL PACKAGING

A convenient way by which antimicrobials activity can be achieved is by the direct incorporation of antimicrobial additive in packaging films (Kerry et al., 2006). Low diffusion rate films are desirable to maintain high surface concentration of impregnated antimicrobials for longer periods. Over a period of release, microbial growth kinetics and antimicrobial activity at the product's surface may be balanced (Appendini and Hotchkiss, 2002). Many methods have been used to incorporate antimicrobials, including extrusion, injection molding, and solvent compounding. Thermally stable antimicrobials may be impregnated into films using thermal polymer processing methods such as extrusion and injection molding. Antimicrobial activity deterioration of antimicrobial films can occur during the polymer extrusion process using high temperature, high shear rates, and thus, high pressure (Brody et al., 2001). The temperature, pressure, and residence time in the extruder have to be calculated mathematically so that the residual antimicrobial activity is known (Han, 2000).

In food packaging, antimicrobial films are obtained by using various methods such as solvent casting technique and surface modification of polymers. Sebastien et al. (2006) proposed a chitosan-loaded polylactic acid film (PLA). Scannel et al. (2000) studied the surface immobilization of bacteriocins (such as nisin) on polyethylene/polyamide materials. The most preferred industrial methods are active films obtained by means of extrusion processes (Nam et al., 2007).

Biopolymers are examples of polymers formed through solvent compounding due to the wide variety of proteins, carbohydrates, and lipids (acting as plasticizers) that form films and coatings. PLA is a biodegradable polymer made from corn following formation of starch and condensation of lactic acid with mechanical and physical properties comparable to other commercially available polymers (Krishnamurthy et al., 2004). Other biodegradable polymers can be derived from petroleum products or may be obtained from mixed sources of biomass and petroleum. Polybutylene succinate, aliphatic polyesters, polycaprolactone (PCL), and polyvinyl alcohol are examples of petroleum-based biodegradable polymers (Jarerat and Tokiwa, 2001). Nobile et al. (2009) reported that PCL-based material incorporated with lysozyme was the most effective antimicrobial system compared to that containing lemon extract and thymol. Solvent compounding may be a more suitable method for incorporating heat-sensitive antimicrobials into polymers (Ishitani 1995; Appendini and Hotchkiss, 1997); in which case both the antimicrobial and the polymer need to be soluble. These polymers as well as their combinations are soluble in water, ethanol and other solvents compatible with antimicrobials (Appendini and Hotchkiss, 2002). Heat sensitive antimicrobials can also be coated onto the material after forming or are added to cast films. Edible coatings and films have a variety of advantages such as biodegradability, edibility, biocompatibility, aesthetic appearance, and barrier properties against oxygen and physical stress (Kerry et al., 2006). Such edible coatings and films can be prepared from polysaccharides, proteins and lipids. Edible coats could help reduce the problem of moisture loss during storage, surface microbial load, and rate of rancidity and restrict volatile flavor loss and foreign odor pick-up (Kerry et al., 2006).

BREAD MICROFLORA AND ANTIMICROBIAL PACKAGING FOR BREAD

Fungi are the most common spoilage organisms in baked products such as bread; the shelf life without preservatives is

3–4 days at ambient temperature (Nielsen and Rios, 2000). The most common fungi are *Penicillium commune, P. solitum, P. corylophilum, P. discolours, P. palitans, P. polonicum, Pichia anomala, A. flavus, P. roqueforti,* and *Eurotium* species. The yeast known as chalk moulds are very important spoilers of bread. The most important species is *Endomyces fibuliger* (Nielsen and Rios, 2000). In wheat bread *Penicillium commune, P. solitum, P. Corylophilum, and Aspergillus flavus are* the most dominant. *P. Roqueforti, P. Corylophilum, and Eurotium* species are more dominant in rye bread (Lund et al., 1996). The rational of incorporating antimicrobials into the packaging is to prevent surface growth in foods where a large portion of spoilage and contamination occurs. Table 5 shows bread spoilage organisms, their habitats, and toxicity.

Low-density polyethylene (LDPE) was developed in 1936 by high pressure polymerization process of ethylene to LDPE (0.915–0.94 gcm^{-3}) resulting in a low molecular weight polymer. Linear LLDPE is a copolymer of ethylene (Piringer and Baner, 2008). LLDPE has a higher tensile strength and higher impact and puncture resistance than LDPE and also very flexible. LLDPE is more difficult to process than LDPE; has lower gloss and a narrower heat sealing range (Anon, n.d).

Sorbates incorporated into thin films diffuse slowly from the film to the surface of the food thus providing surface protection of the food. Films with low diffusion rates are more desirable since they maintain high concentrations of sorbates for longer periods (Vojdani and Torres, 1989). Sorbates are incorporated at 0.1–5% w/w of the packaging material, particularly films. Potassium sorbate (1%) in a LDPE film is reported to inhibit the growth of yeasts on agar plates (Vermeiren et al., 1999). Sorbates could be incorporated into polymers in the melt or by solvent compounding. Thermal processing methods such as thin film extrusion may be used with thermally stable antimicrobials (Appendini and Hotchkiss, 2002).

Addition of antimicrobials to packaging materials affects the physical properties such as tensile strength, elongation, burst strength, tearing strength, tearing resistance, stiffness, and oxygen and gas permeability. The process, machine ability of the packaging materials is then affected. Water vapor permeability, wet ability, water absorptiveness, grease resistance, brightness, haze, gloss, and transparency is also affected (Han, 2000). The antimicrobials position themselves in the amorphous structural regions of the polymer but will not affect the polymer mechanical strength if it is a properly antimicrobial designed system (Han, 2000). If an excessive amount of antimicrobial agent is mixed into the polymer, the amorphous region can be saturated and the additive can interfere with the polymer blend which affects processability, physicomechanical properties, and optical properties (Han, 2003). Before and after incorporating potassium sorbate into LDPE film Han and Floros (1997) reported no change in the tensile properties. By increasing the potassium sorbate concentration the transparency of the LDPE films decreased (Han and Floros, 1997). Transparent films are highly desired for food packaging for improved product visibility (Wang et al., 2005).

Volatile antimicrobial substances from spices have proved to be efficient at controlling fungal spoilage of bread. Suhr and Nielsen (2003) reported that the antifungal effects of essential oils on rye bread spoilage fungi depended on the application method. Large phenolic compounds such as thymol and eugenol (thyme, cinnamon, and clove) has best effect when applied directly to the medium. Smaller compounds such as most ally isothiocyanate and citra (mustard and lemongrass) were most efficient when added as volatiles.

Gutiérrez et al. (2011) demonstrated that cinnamon-based active packaging a suitable alternative for conventional MAP for gluten-free bread based on both microbial and sensory criteria. Allyl isothiocyanate obtained from mustard seed oil has the strongest effect on all test fungi at 3.5 μg/mL gas phase. Potassium sorbate also totally inhibited growth over a wider range of conditions and this would thus be the most suitable weak acid preservative for bakery products. It prevented fungal growth at both pH 4.5 and 6, regardless of a_w levels (080–0.95 a_w). A combination of allyl isothiocyanate and potassium sorbate incorporated into polymers may provide the needed natural antimicrobial protection for bread products. Nielsen and Rios (2000) reported that one way of applying AITC is to pack bread in AITC incorporated film

Table 5 Bread spoilage organisms, habitat, and toxicity

Fungus	Habitat	Mycotoxins
Penicillium commune	Cheese	Cyclopiazonic acid
P. solitum	Hard cheeses, liver pate, meat, dried fish	Unknown
P. corylophilum	Grows on a variety of foods	Unknown
P. discolours	Cheese, nuts, vegetables, apples	Chaetoglobosin A,B
P. palitans	Hard cheeses, bread, meat	Cyclopiazonic acid, fumigaclavine A&B
P. polonicum	Cereals, meat products	Nephrotoxic glycopeptides, penicillic acid
P. roqueforti	Cheese, rye bread, yeast, wood	PR toxin, roqueforti C & isofumigaclavine A&B, Mycophenolic acid
Pichia anomala	Bread	Unknown
Aspergillus flavus	Grows on a variety of foods	Aflatoxin
Endomyces fibuliger	Rye bread	None reported
Eurotium species	Variety of foods	None reported

Adapted from (Mycobank, 2010).

(polyethylene or ethylenevinyalcohol). The AITC will migrate through the film, killing the microorganisms and leaving the product sterile and with a concentration of AITC less than the sensory threshold. However, there is a problem of loss of active volatiles of these components at high processing extrusion temperatures (Nielsen and Rios, 2000).

To reduce the thermal effect of extrusion on the allyl isothiocyanate and sorbate the LDPE resin and the sorbate powders could be mixed, extruded at low temperature and palletized to produce a masterbatch (Han, 1997). The pellets can then be added and blended into the final polymer mix and then extruded into the final LDPE and LLDPE blend containing the correct amount of antimicrobial agent. The double extrusion process results in a homogeneous mix of the antimicrobial agent. This then results in a uniform controlled release of the antimicrobial agent (Han, 2000). The resulting film can then be coated with allylisothiocyanate or added to cast edible films (Appendini and Hotchkiss, 2002). Moisture scavenger may be included to reduce the effect of moisture condensation on the bread product.

CONCLUSION

Incorporation of volatile antimicrobial substances from spices and herbs into packaging materials may provide alternative effective ways of prolonging the shelf life of bread without the use of chemical preservatives. Antimicrobial packaging is thus economical and more favorable for bread as (1) special packaging machines are not required; (2) no gases required as in MAP; and (3) high-barrier material requirement in MAP is not necessary. A combination of AITC and sorbate may solve this problem. Adoption of this route will enable the food industry to fulfill the demands of minimum changes in food quality and maximum security.

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