Use of Natural Antimicrobial Agents in Food Packaging

Siddhesh Khade

T.Y.B.Tech.
Foods Engineering & Technology Department

Abstract

Antimicrobial packaging is a novel packaging technology that aims to prolong the shelf-life of food by inhibiting the growth of contaminating microorganisms without significant adverse effects on organoleptic and nutritional properties [1]. Interest in natural antimicrobials (AMs) has expanded in recent years in response to consumer demand for greener additives. During the last two decades, these AMs have been investigated for their practical applications. The article reviews the naturally occurring AMs that can be used in food grade packaging, their mechanism of action, and how they can be used in food grade packaging.

Keywords: Naturally occurring AMs, Antimicrobial packaging, Antimicrobial peptides, Chitosan.

1. INTRODUCTION

This review introduces the background of food packaging and, in particular, antimicrobial (AM) packaging (a novel packaging technology that aims to prolong the shelf-life of food by inhibiting the growth of contaminating microorganisms). It also examines the advantages of use of naturally occurring AM agents in food packaging.

1.1 Background

In food manufacturing, "packaging" is a discipline of a post-production process, where a product is enclosed in a container (or wrapping) for many purposes, including protection, transportation, distribution, storage, retailing and end-use [1]. There are typically three circumstances where a food package interacts with the environment. Firstly, the package is in physical contact with its surroundings that may cause damage to the packaged food. Secondly, it is in contact with the ambient surroundings which can include gases, water, light, temperature, microorganisms and pests. Thirdly, it can affect potential purchasers with its appearance and the information on it. However, other than the interaction of food package with its environment, there is another category of food packaging i.e. Active Packaging which is designed to have the ability to interact with the packed constituents [2]. Active Packaging is an innovative concept that can be defined as a mode of packaging in which the package, the product, and the environment interact to prolong shelf life or enhance safety or sensory properties, while maintaining the quality of the product [3].

AM packaging is a type of active packaging, where the package is designed to release active agents to inhibit the growth of microorganisms inside the package. This type of packaging is in contrast with that involving the addition of chemical preservatives directly into the food matrix, where an excess amount of these synthetic additives is believed to be of concern. For the consumer, it seems to be safer when active agents are indirectly integrated in the food package and released into the food product thereafter [4].

1.2 Advantages of Using Naturally Occurring AM agents in Food Packaging

Consumers tend to accept products to which naturally occurring substances have been added more than those containing synthetic agents[5]. This trend subsequently draws many researchers to integrate natural AM agents into food packaging materials. In addition to improving the shelf life and safety of foods, natural antimicrobial agents may allow novel food products with enhanced quality and nutritional properties to be introduced to the market. This article reviews several research papers about the use of natural AMs in foods packaging & are enlisted in table 1.

Table 1. Advancements in AM Packaging having Natural AMs.

No.	Developments	Ref.	Year
1.	Development of a cheese wrapping paper containing sorbates as antifungal agents using carboxy methyl cellulose (CMC) as a binder	25	1973
2.	Garlic oil incorporated into chitosan film showed AM activity against E. coli, S. aureus, S. typhimurium, L. monocytogenes, B. cereus	20, 21	2005
3.	Edible apple- & tomato- based films, prepared from their slurries & containing low levels of plant essential oils (oregano, cinnamon, lemongrass) or their major constituents (carvacrol, citral & cinnamoldehyde) induced reductions in E.coli	22	2006, 2007
4.	Films made of partially hydrolyzed sago starch & alginate mixture containing lemongrass oil inhibited E. coli	23	2007
5.	Films with a 90/10 blend ratio of chitosan & polyethylene oxide exhibited strong AM activity	24	2007

6. Edible apple film wraps containing plant AM inactivate foodborne pathogens on meat & poultry products	05	2009

2. Food Spoilage by Microorganisms

Excessive growth of microorganisms can change the taste, aroma, appearance and texture of foods and may even causes health hazards. Most foods can be unsafe to consume when they are contaminated with foreign microorganisms and unfortunately, consumers are unable to recognize foods as spoiled until the items start presenting an unsatisfactory taste, odor appearance or texture. The subsequent consumption of contaminated foods can cause food poisoning and other food-borne diseases [6]. Adequate hygiene is very important in food processing in order to prevent or minimize contamination by microorganisms.

Food manufacturing in aseptic conditions is very expensive and some food industries cannot afford it. Therefore it is accepted that most food products contain microorganisms to some level. As a result, it is intended that these food products should be consumed within a limited time while the number of microorganisms is still at an adequate level for consumption.

Microorganisms from a variety of sources can contaminate the surface of solid foods and penetrate into the bulk of the food. In liquid foods, the contaminating microorganisms can spread easily whereas in solid foods the contamination generally remains on the surface of the food [7].

3. Potential Natural AM Agents

The potential AM agents of natural origin can be classified as Plant origin AM agents and Animal origin AM agents.

3.1 AMs of Plant Origin

Further classification of AMs of plant origin, examples and their mechanism of action is given in Table 2.
table 2
3.1.1 Factors affecting AM activity of plant AMs:-
AM activity of Essential Oils (EOs) is influenced by a number of factors including botanical source, time of harvesting, stage of development, and method of extraction. For example, Satureja EOs obtained during the flowering period was the most potent with bactericidal properties [8]. The composition, structure as well as functional groups of the oils play an important role in determining their AM activity. Usually compounds with phenolic groups are the most effective. Most studies on AM efficacy of EOs have been conducted in vitro using microbiological media. Consequently, there is less understanding related to their efficacy when applied to complex food systems. Key areas requiring further knowledge for optimized application of natural AMs in food include targeting the microorganisms of concern, the intelligent use of combination of AMs to provide a synergy of activity, matching the activity of the compounds to the composition, and processing and storage conditions of the food [1].

Plant EOs of thyme, clove, and pimeto show activity against Listeria monocytogenes and were found to be highly effective in peptone water. However, when the EOs are applied in a food system, efficacy of EOs reduces due to interaction with components. In general, higher concentrations of EOs are required in foods than in laboratory media [9]. Combination of EOs can minimize the application concentrations required, thereby reducing any adverse organoleptic impact; however, their application for microbial control may also be affected by food composition [1]. The AM efficacy of EOs is found to be a function of ingredient manipulation, for example, the AM activity of thyme is increased in high protein concentrations, concentrations of sugars above 5% on the microbial growth medium did not reduce microbial growth efficacy, and high potato starch concentrations decreased the EO antimicrobial activity of oregano and thyme on L.monocytogens in food model systems [1]. Finally, low pH values (of average range of 5) seemed to have the highest impact on the increase of the AM effect of the EOs of L.monocytogens. Low pH values appear to increase the hydrophobicity of the EOs,

Table 2. Major Classes of AM Compounds from Plants

Class	Subclass	Example(s)	Mechanism
Phenolics	Simple phenols	Catechol	Substrate deprivation
		Epicatechin	Membrane disruption
	Phenolic acids	Cinnamic acid	
	Quinones	Hypericin	Bind to cell wall, complex with cell wall, inactivate enzymes
	Flavonoids	Chrysin	Bind to cell wall, Complex with cell wall
	Flavones		
		Abyssinone	Inactivate enzymes
			Inhibit HIV reverse transcriptase
	Flavonols	Tobacco	?
	Tannins	Oligomeric	Bind to proteins
			Bind to cell walls
			Enzyme inhibition
			Substrate deprivation
			Complex with cell wall
			Membrane disruption
			Metal ion complexation
	Coumarins	Warfarin	Interaction with eucaryotic DNA (antiviral activity)
Terpenoids, essential oils		Capsicin	Membrane disruption
Alkaloids		Berberine	Intercalation into cell wall and/or DNA
		Piperine	
Lectins and polypeptides		Non-immune-specific agglutinins	Block viral fusion or adsorption
		Ribosome	Formation of disulfide bridges

Reference:-[26]

consequently enabling easier dissolution in the lipids of the cell membrane of target bacteria [1].

Accordingly, the challenge for the practical application of EOs is to develop optimized low dosed combination to maintain product safety and shelf life, thereby minimizing the undesirable flavor and sensory changes associated with the addition of high concentrations of EOs.[1].

3.2 Animal Origin AM Agents

AMs of animal origin are often evolved as host defense mechanisms of animal body. Major AMs of animal origin include Chitosan, Antimicroal Peptides (AMPs).

3.2.1 Chitosan

It is a natural biopolymer obtained from the exoskeletons of crustaceans and anthropods, and is known for its unique polycationic nature. It is used as active material for its antifungal activity, and AM activity. Low molecular weight chitosan is effective for controlling growth of microorganisms [10]. The strong antibacterial activity of chitosan was also observed against S. aureus, while its molecular weight appeared to be significant parameter defining its activity [1].

3.2.2 Antimicrobial Peptides (AMPs)

AMPs are natural AM agents used against a wide variety of pathogens. They are generally found in the tissues which are most exposed, such as skin, eyes and lungs of animals including lymph of insects. The active compounds are also of microbial origin [11].

3.2.2.1 AMPs of microbial origin

AMPs derived from microbial source are typically made by bacteria. These are low molecular weight AMPs that can inhibit other bacteria and are also called "bacteriocins" [12]. The amino acid sequence of some bacteriocins is summarized in Table 3.

Table 3 : Amino Acid Sequence of Some Microbial Derived

AMPs	Amino Acid Sequence *
Nisin	ITSISLCTPGCKTGALMGCNMKTATCHCSIHVSK
Pedioocin PA1	KYYGNGVTCGKHSCSVDWGKATTCIINNGAMAWATGGHQGNHKC
Leucocin A	KYYGNGVHCTKSGCSVNWGEAFSAGVHRLANGGNGFW
Sakacin P	KYYGNGVHCGKhSGCTVDWGTAIGNIGNNAAANWATGGNAGWNK
Bacteriocin 31	ATYYGNGLYCNKQKCWVDWNKASREIGKIIVNGWVQHGPWAPR
Enterocin A	TTHSGKYYGNGVYCTKNKCTVDWAKATTCIAGMSIGGFLGGAIPGQC
Enterocin P	ATRSYGNGVYCNNSKCWVNWGEAKENIAGIVISGWASGLAGMGH

* Positive charged residues are marked in bold and underlined
Reference:- [18]

3.2.2.2 AMPs of Animal Origin

An AMP, Dermaseptin S4, extracted from the skin of Phyllomedusa tree frogs when incorporated in a corn starch-based coating and applied on cucumber, showed an inhibitory effect against moulds and aerobic bacteria [13]. The amino acid sequence of some animal derived AMPs is summarized in Table 4.

Table 4 : Amino Acid Sequence of Some Animal Derived AMPs.

AMPs	Amino Acid Sequence *
Magainin	GIGKFLHSAKK FGK AFVGEIMNS
MSI-78	GIGKFLKKAKKFGKAFVKILKK$_{CONH2}$
PR-39	RRRPRPPYLPRPRPPPFFPPRLPPRIPPGFPPRFPPR FP
Spheniscin	SFGLCRLRRGFCAHGRCRFPSIPIGRCSRFVQCCRR VW
Pleurocidin	GWGSFFKKAAHVGKHVGKAALHTYL
Dermaseptin S4	ALWMTLLKKVLKAAAKALNAVLVGANA
K4S4 (1-14)	ALWKTLLKKVLKAA$_{CONH2}$
Cecropin P1	SWLSKTAKKLENSAKKRISEGIAIAIQGGPR
Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES
Clavanin A	VFQFLGKIIHHVGNFVHGFSHVF
Curvacin A	ARSYGNGVYCNNKKCNVNRGEATQSIIGGMISGWASGLAGM

* Positive charged residues are marked in bold and underlined
Reference:- [18]

3.2.3 Mechanism of AM Action

The mechanism of action of AMPs seems to involve multiple targets. The plasma membrane is the main target; however, recent studies intracellular targets at least for some peptides. Although most AMPs act by non specific methods, they often display some selectivity between different microorganisms, for example, Gram negative bacteria compared with Gram positive bacteria and susceptibility of fungal cells compared with other eukaryotic cells [13]. AMPs can assume amphipathic structures, which are able to interact directly with the microbial cell membrane rapidly disrupting the membrane in several locations, resulting in leaching out of vital components. Previous studies carried out on the mechanism of action of pleurocidin revealed that it exhibits strong membrane translocation and pore formation ability, reacting with both neutral and acidic anionic phospholipid membranes [13]. Lipids inactivate microorganisms mainly by disruption of bacterial cell wall or membrane, inhibition of intracellular replication, or inhibition of an intracellular target. Monoacylglycerol lowers the heat resistance of certain bacteria and fungi; therefore, they may find application in reducing the required heat treatment for certain foods. Lysozyme hydrolyses the ß-1, 4- glycosidic linkage in sugar polymers such as N-acetylmuramic acid and N-glucosamine linkages found in bacterial peptidoglycan [13].

3.2.4 Factors Affecting AM Activity

Various factors can impact the antimicrobial efficacy of bacteriocins. These include the emergence of bacteriocin resistant bacteria, conditions that destabilize the biological activity of proteins such as proteases or oxidation processes, binding to food components such as fat particles or protein surfaces, inactivation by other additives, poor solubility, and uneven distribution in the food matrix and/or pH effects on bacteriocin stability and activity [1]. The application of bacteriocins in combination with other preservation hurdles has been proposed to reduce the selection for resistance to bacteriocins in target strains and/or to extend its inhibitory activity to Gram negative species. Interactions between bacteriocin and the food matrix may result in a decrease in the efficacy of the bacteriocin [1]. The combination of bacteriocins with other minimal or nonthermal preservation technologies may prove useful for practical applications. This approach is of value for the control of Gram negative bacteria as their outer membrane acts as an efficient barrier against hydrophobic solutes and macromolecules, such as bacteriocins. [1]

4. Functioning Modes of Antimicrobial Packaging

AM packaging can be categorized into two types: one function by a nonmigratory mechanism and the other works by releasing the active agents into food. For the non-migratory type, the active substances are immobilized onto the package surface and the inhibition happens when microorganisms contact with these active substances [13]. This type of packaging needs the circulation of food in the package, which normally is practical with liquid food. For the migratory AM packaging, there are two subcategories i.e. the packages that are incorporated with non-volatile active agents and the packages with volatile substances [5]. Figure 1 shows the schematic diagram of three functioning modes of AM packaging described above. The AM film made of polypropylene (PP), ethylene vinyl alcohol (EVOH) and polyethylene (PE) containing cinnamon extract was observed to remotely inhibit the activity of fungi. In this case, the vapors of the AM agents released from the AM films could create a protective AM atmosphere and direct contact was not necessary [13].

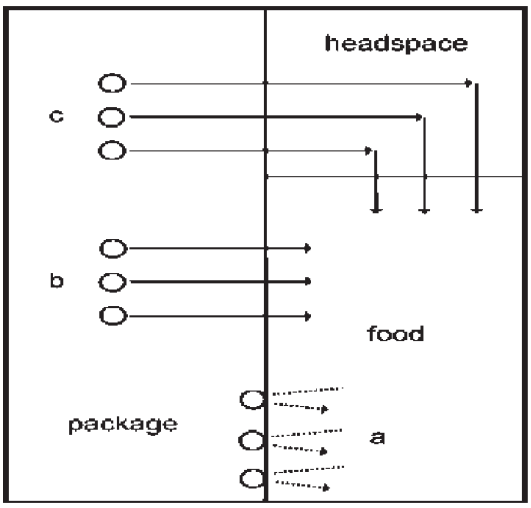

Figure 1 : Functioning modes of AM packaging: (a) Non-migration; (b) Nonvolatile Migration; and (c) Volatile migration.
Reference:- [19]

5. Controlled Release

AM agents that are safely used in food products are also assumed to be safe when incorporated in packaging materials. Rather than adding a high dose of AM agents directly into food, the application of AM packaging offers potentially the advantage of a slow release of the AM agents onto the food surface and retains the required concentrations to inhibit microbial growth for longer periods [14]. The continuous slow release should be advantageous for resealable packages for consumers as they may be opened and closed numerous times [15]. The release of AM agents from polymeric packaging materials is claimed to be systematically controllable [16]. (See figure 2).

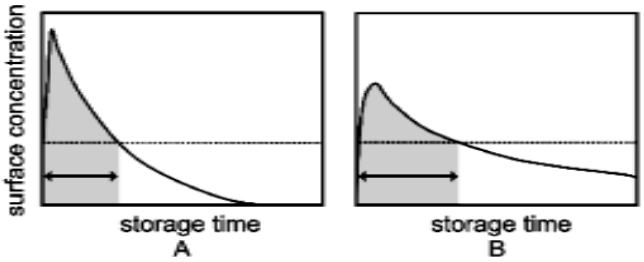

Figure 2 : Release Profiles of AM Agents from Different AM Packaging Films.

Figure 2 shows AM agent release profiles from three different AM packaging films.
System A and B show the release from a monolithic film or so-called "matrix model".
System A demonstrates a rapid AM agent release featuring a high solubility in food
media while system B shows a slower release due to a lower solubility in food. The

6. Conclusions & Future Trends

Reported studies have demonstrated that natural AM agents described in this review may offer unique advantages for food processing. In addition to improving the shelf life and safety of foods, natural AM agents may allow novel food products with enhanced quality and nutritional properties to be introduced to the market. The applications of natural AM agents are likely to grow steadily in the future because of greater consumer demands for minimally processed foods and those containing naturally derived preservation ingredients. More complex considerations arise for combination of technologies, particularly with respect to optimization of practical application. Intelligent selection of appropriate systems based on detailed, sequential studies and quantitative approaches to evaluate the efficiency of AMs is necessary. The reported effectiveness of natural plant extracts suggests that further research is needed in order to evaluate their AM activity and potential side effects in packaged foods. An additional challenge is in the area of odor/flavor transfer by natural plant extracts to packaged food products. Thus, research is needed to determine whether natural plant extracts could act as both an AM agent and as an odor/flavor enhancer. Moreover, in order to secure safe food, amendments to regulations might require toxicological and other testing of compounds prior to their approval for use [17].

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