Asian J. Adv. Basic Sci.: **2017, 5***(2)*, 54-59
ISSN (Print): 2454 – 7492
ISSN (Online): 2347 – 4114
www.ajabs.org

Incorporation of Natural Antimicrobials in Edible Films for Food Preservation: A Review

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(Received 18 Aug, 2017; Accepted 04 Sept, 2017; Published 23 Sept, 2017)

ABSTRACT: Development of edible films proves to be a potential alternative for conventional synthetic plastic based preservation employed in food packaging industry, since the use of chemical additives has raised questions for the consumption of such preserved foods. Application of antimicrobial compounds from natural resources such as plants would be better substitutes when incorporated into bio- degradable edible films thus enhancing their safety. Several compounds which have been proposed to have such antimicrobial activity against food borne pathogens are found to be extracts or essential oils from plant origin. Studying the physicochemical properties of such compounds incorporated in edible films will provide a better insight in improving the mechanical strength and stability of the films and enable their successful development. This review focuses on the use of edible coatings as carriers of natural antimicrobials which can be used to preserve fruits and vegetables for increasing their shelf lives.

Keywords: Edible films; essential oils; food preservation; natural antimicrobials and shelf- life.

INTRODUCTION: Food contamination and spoilage by microbial contamination still pose to be an important public health and economic concern for the human society. Among many strategies to inhibit the growth of undesirable microorganisms, the use of chemical agents that exhibit antimicrobial activity are prevalent. These chemicals may be either synthetic compounds intentionally added to foods or naturally occurring and biologically derived substances. But, advances in analytical methods have raised questions concerning the safety of such synthetic compounds.[1] Hence, the consumers prefer natural additives including antimicrobial agents incorporated into food products.[2] Several research groups have documented the activity of natural antimicrobial compounds isolated from fruits, vegetables, grains, herbs and spices which can be incorporated.[3] Packaging plays a fundamental role for food preservation by maintaining quality, to protect from microbes and for preparing the product for commercial handling. Since, the final disposal of synthetic films results in additional recycling costs and ecological problems, use of biodegradable edible films are being preferred.[4] This review focuses on the importance of biodegradable edible films which are incorporated with natural antimicrobials.

EDIBLE COATINGS AND THEIR COMPOSITION: Edible coatings are thin layers of edible material applied to the product surface to control moisture transfer and gas exchange. Biopolymers such as proteins, lipids and polysaccharides can be used for the formation of edible films and coatings.[5] An edible coating is a thin layer of edible material formed as a coating on a food product, while an edible film is a preformed thin layer, made of edible material, which can be placed on or between food components.[6] They are applied directly on the food surface by dipping, spraying, or brushing to create a modified atmosphere.[7] As the edible films are ultimately consumed, the material used for the preparation of edible films and coatings should be generally regarded as safe (GRAS) approved by FDA and must conform to the regulations that apply to the food product concerned[8]. Edible and biodegradable coatings must meet a number of special functional requirements, for example, moisture barrier, solute or gas barrier, water/ lipid solubility, color and appearance, mechanical characteristics, less toxic, etc. The effect of coatings on fruits and vegetables depends greatly on temperature, alkalinity, thickness and type of coating, and the variety and condition of fruit and vegetable.[9]

Edible coatings can be produced from materials with film forming ability. The solvents used can be water, alcohol, mixture of water and alcohol, or a mixture of other solvents. Edible coatings may be composed of polysaccharides, proteins, lipids, and composites. A combination of these often gives best results. Edible coatings can also be made from a variety of polysaccharides.[10]

Plasticizers, antimicrobial agents, minerals, vitamins, colors, or flavors can be added in this process, adjusting the pH and /or heating the solutions may be done for the specific polymers to facilitate dispersion.[11]

A mixture of sucrose fatty acid esters (SFAE), sodium carboxy methyl cellulose, and mono- and diglycerides was developed as an emulsifier coating material which retards fruits' ripening. SFAE mixtures have been commercially available since 1980's for coating fruits and vegetables under the trade names "TAL Prolong" and "Semper fresh".[12]

STARCH BASED BIOPOLYMERS*:* The principal polysaccharides of interest for material production are cellulose, starch, gums, and chitosan. Linear structure of some of these polysaccharides, for example, cellulose, amylose (a component of starch), and chitosan renders their films tough, flexible, transparent, and resistant to fats and oils. More complex polysaccharides are produced by certain fungi and bacteria such as xanthan, curdlan, pullan and hyaluronic acid, and these will receive more interest in the future.[13]

Biodegradable polymers from agro- resources are considered as alternatives to non- degradable polymers which play an important part in food packaging industry.[14] Starch, in its thermoplastic form is becoming the most preferred form of a biodegradable packaging raw material.[15] Starch varies in size (2-100 µm), size distribution, shape and chemical composition and also according to the sources which may be potato, maize, rice wheat, cassava, waxy maize, amylo maize etc.[16] Starch is mainly comprised of two structurally distinct α- D glucan components of which, amylose is linear and amylopectin is highly branched. The source and the amylose content have an influence on the crystalline structure of starch, but amylopectin forms the dominating crystalline component in native starch.[17]

Water acts as a destructuring agent for starch and therefore it requires water dispersion and partial or complete gelatinization to be used as a polymer matrix.[18] The brittleness of the thermoplastic starches are overcome by using plasticizers to make them flexible for film applications.[19, 15] The most frequently used plasticizer in starch based films are polyols, such as sorbitol and glycerol which prevent cracking of the film during handling and storage.[20] Glycerol is a natural plasticizer, which reduces intra and inter- molecular hydrogen bonds thereby reducing film brittleness.[21] Further, the water vapour permeability, film lightness and water sorption was found to increase with increase in glycerol content by 30% in a study done by Farhanaky et al in 2012.[22] This improvement in the physical and mechanical properties of films is imperative in assessing the applicability of starch based edible films in food and pharma industry.

Like cellulose, starch nano crystals (SNCs) can be produced by acid hydrolysis. The use of SNC has gained interest recently as the addition of nanofillers allows to present a large surface. This in turn presents a huge interfacial area between the filler and polymer matrix.[23] These bio- nanocomposites developed from the hydrolysis of waxy maize starches are rich in amylopectin.[24] Biobased edible films with native potato starch combined with glycerol, catechin and starch nano crystals have been successfully developed.[14] Their mechanical performance and ability to disintegrate under compost conditions have been found to confirm their use as biodegradable edible films for food products.

PROPERTIES FOR CHARACTERIZING EDIBLE COATINGS: The properties of edible coating depend primarily on molecular structure rather than molecular size and chemical constitution. The primary requirement for any edible film and coating is that the coating should be water-resistant so that it remains intact and covers a product adequately, when applied. It should not deplete oxygen or build up excessive carbon dioxide thus maintaining effective gas exchange.[11]

Hydrophobic substances such as resins, waxes, or some insoluble proteins are better moisture barriers, but water-soluble hydrocolloids like polysaccharides and proteins usually impart better mechanical properties (tensile strength and elongation at break) to edible films and coatings than do lipids and hydrophobic substances.[25]

The physico- chemical and barrier properties of edible coatings which in turn contribute to the effectiveness and functionality of an edible coating are very essential in characterizing the final coated products. Hence, the parameters employed for the analysis of the coated products are discussed under the following categories.

Appearance and color: The coatings applied onto foods generally cause a change in color. Therefore the chromatic parameters such as luminosity, chroma and hue from reflection spectra are analysed for a coated food and compared with an uncoated sample.[26] The gloss units are obtained by measuring with the flat surface glossmeter. The use of this method is limited as it is often employed for planar samples of coated peels.[27]

Water and acid solubility: When films are hydrophilic in nature they tend to absorb more water which in turn increases the WVP (Water vapour permeability) and film solubility properties. Fakhouri et al. (2012) have proved this by increasing the gel concentration in the formulation.[28] In the same trend, the solubility analysis in acid by using HCl helps to simulate the conditions in the mouth meaning that the coating would be completely dissolved when consumed.[29]

Thickness and microstructure: It is necessary to measure the thickness of the edible coatings as they directly determine other properties such as permeability to gases. The usual measurements are done with a hand held micrometer. But once they are applied to the food, it is difficult to measure accurately and so quantifications of the Surface Solid Density (SSD) has to be done.[30, 31.] The mass fraction of the solids (X_s) and the mass of the coating solution adhered to the fruit surface ($M_{F\ a}$) has to be considered for the determination. Moreover, the estimation of the average sample area (A_s) has to be carried out using image analysis techniques or volumetric and surface area measurements. One of the most promising and recent technique used for finding film thickness is the use of spectroscopic ellipsometry. The principle of this method is based on the determination of the complex reflectance ratio of the reflection co-efficient of light, which are polarized paralelly and also perpendicularly to the plane of incidence of light.[32]

Water vapour permeability (WVP): The capacity of the films to work as barriers to water vapour is dependent on the relative humidity and temperature of the environment. Hydrophobic compounds such as lipds (bees wax, carnauba, candelilla and milk fat) included in the formulation help in reducing water loss in fresh cut produce.[33] The WVP of films are generally measured according to a ASTM E96 standard test method.[34] No film with a much lower WVP has been developed till date, when compared to artificial polymer plastic films, which is a problem faced in coated fresh- cut produce.[35]

Gas permeability: The gas permeability of the coatings are obtained by measuring the internal composition of the coated food, in terms of O_2 and CO_2 concentration, some volatile compounds such as ethanol and acetaldehyde. The samples are usually obtained from the core of the fruit by using syringe and then injected in a gas chromatograph column.[27] In contrast to WVP, the lower oxygen permeability of an edible coating is much advantageous than using a conventional plastic film.[31]

Mechanical properties: Edible films are also evaluated for their mechanical properties such as tensile strength, elongation and elastic modulus. Tensile strength (TS) gives an idea of the maximum stress developed in a film and Elongation (E) gives an idea of about the capacity to which the film can be stretched.[36] With increase in relative humidity, the tensile strength decreases while elongation increases which is based on the water content in the coating.[37]

Microbial analysis: Once a food is coated, the atmosphere is modified, which may inhibit the growth of microbes responsible for spoilage but encourages the growth of pathogens. The growth of *C. botulinum* is one such case. Thus extending the shelf life of foods by coatings gives a chance for pathogens such as *L. monocytogenes, Yersinia enterocolitica*, and *Aeromonas hydrophila* even under refrigerated conditions. [38]

APPLICATION OF EDIBLE COATINGS ON FRUITS: Edible coatings can be applied on whole and fresh-cut fruits and vegetables. Research and development efforts focus on improving the functional characteristics of the coatings and these are based on the properties of the fruit to be preserved or enhanced. [31] Fruits and vegetables that are coated as whole include apple, kinnow, grapefruit, honey dew, avocado, orange, lime, peach, pear, strawberry and lemon. Vegetables that are used for coating as whole are cucumber, bell pepper, melons, tomato.[39]

The mechanism involved in edible coating may differ from controlling of moisture transfer and fruit metabolism to the release of antimicrobial substances, thus extending fruit shelf-life. [39] Minimally processed fruits which have been cut or peeled are also being preserved with edible coatings which have a quality and freshness similar to the fresh fruit.[40] Cut fruits have liquid exudates and remain wet for longer periods, which reduces the adherence of edible coatings on minimally processed fruits. Therefore the drying of the coatings on these fruits is found to be much slower. Such fruits are coated with pectin first and then osmotically treated to obtain higher dehydration efficiency which are then edible coated.[41, 42] Such coatings have been successful in strawberries with different concentrations of sodium alginate, carrageenan or guar gum solutions prior to osmotic dehydration.[43]

PLANTS AS PRESERVATIVES: The side effects caused by the use of the chemical preservatives and synthetic antimicrobial agents have been paved the way for potentially active agents, from the natural sources, such as from the plant sources. Plants contain more of antimicrobial agents and screening of those antimicrobial agents has gained much importance for the development of quality and nutritionally rich foods.[44] Moreover, consumers accept products which have coatings incorporated with naturally occurring substances which draws research to be focused on incorporation of natural antimicrobial agents in food packaging materials.[45] Natural compounds which have been widely used include, plant derived secondary metabolites such as essential oils and phytoalexins, chitosan, nisin or lysozyme and these are declared as "green label" products.[46]

Among these, essential oils are extensively used in biodegradable edible films and coatings where they reduce water vapour permeability of hydrophilic films efficiently due to their lipidic nature. [47] Cassia, clove, garlic, sage, oregano, pimento, thyme, rosemary, lemongrass, scutellaria and forsythia are rich sources of plant secondary metabolites.[48] Du et al. (2009) developed edible films incorporated with oregano and garlic essential oils which were found to be effective *against E. coli, Salmonella enterica* and *L. monocytogenes*.[49] Moreover, films based on pectin and apple, carrot and hibiscus were incorporated with carvacrol and cinnamaldehyde and had a pronounced antimicrobial activity.[50, 51] Addition of 0.3% w/v lemongrass in alginate based edible coatings has been used on fresh cut pineapples to increase shelf life and maintain its quality.[52] Incorporation of rosemary extracts have been studied by Takala et al. (2013) where the incorporated films had good inhibitory activity against *Listeria monocytogenes*, *E. coli* and *Salmonella typhimurium* on broccoli.[53] Essential oils from the leaves of *Croton blanchetianus* also form an alternative source of potentially natural antimicrobial agents which can be applied for food preservation.[54] Antimicrobial efficacy of the extracts from Mexican lime and oregano have also been found to be effective in reducing bacterial pathogens such as *Shigella, Salmonella* and *E. coli* which are associated with contaminated green leafy vegetables.[55] Starch based edible films containing Origanum oil (*Thymus capitatus*) have shown greater inhibitory effects against gram negative food borne pathogenic bacteria.[56] *Punica granatum* and *Rhus coriaria* extracts have also been identified as natural antimicrobial agents which can control food related bacterial biofilms.[57]

CONCLUSION: Since there is a high demand to protect highly perishable foods such as fresh fruits, minimally processed fruits and vegetables, new technologies are developed to provide safety in food industry. Edible coating technology proves to be a promising strategy in food preservation as it is safe. Studies on the film properties when incorporated with plant extracts possessing antimicrobial activities are a major area of focus today. Further, nanostructured and multilayered coatings are also developed which makes the coatings tailor made in future. Specific functional enhancements are possible if the selection of the film forming components and active ingredients are chosen appropriately with the objective to develop a successful preservative coating.

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