

**Protection of Seafood by Edible Films and Genetic Modification of
Protective Culture**

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Abstract

Seafood is a good source of nutrients like protein, fats, vitamins and many other micronutrients. The texture of seafood makes it more vulnerable to degrade due to microorganisms. As with the passage of time, seafood consumption increased seafood industries tries to overcome this problem and to extend the shelf life of seafood by controlling the growth of microorganisms and improve the quality of seafood by retarding different metabolic and enzymatically reactions which leads fast towards degradation. For this purpose scientists use different techniques like protective culture made from a starter culture of microorganisms and some protective culture is made from the genetic changes in the genome of microorganisms with the help of recombinant DNA technology. Scientists used edible films to cover the seafood products to prevent the interference of the external environment with food product so it can store for long without any deterioration. This review will cover different microbial protective culture, genetically modified protective culture and about properties of the coating or edible films on seafood products.

Keywords: Seafood, Genetic modification, Micro-organisms and shelf life

Review article

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INTRODUCTION

Seafoods are the water animals having habitat in different water sources from small rivers to ponds and from lakes to saltwater, which are used as a rich nutrient source for human health. Fish is identified as a full healthy diet containing various valuable nutrients like protein, fatty acid, vitamins, minerals, micronutrients, Omega 3- fatty acids and taurine (Basurco et al., 2014). Fatty acids are the important health constituent especially in children for brain and heart development, therefore, seafood should be included in our daily food. People interest in seafood across the globe increased day by day even nutritionists also suggest the intake of seafood in daily life. As consumption increased it is notified that seafood is more vulnerable to decompose by various microorganisms and due to different chemical and biological reactions (Dalgaard et al., 2006; Mejlholm et al., 2008). Instead of the highest nutritionist value fish also can cause many foodborne diseases names.

Seafood has the main problem to become deteriorate easily rather than other foods because of its structure, to overcome this problem different methods are used. Conventionally pathogenic microorganisms were controlled by steam and drying but now various new methods are developed like there is a technique known as bio protection in which live microorganisms are added to food to control the risk of food toxicity. These microorganisms are got from fermentation cultures and are called protective cultures (Hansen, 2002). It is a good practice to inhibit the degradability of food. These protective cultures also act as a good indicator of risk under bad conditions of food such as abnormal temperature, bad handling conditions and biochemical reactions of food after degradation.

Some chemical methods are also used for the preservation and to increase the shelf life of food but customers prefer natural product over the artificial in such case biopreservation method become more reliable and considerable for use than other techniques (Rodgers, 2001; Calo-Mata et al, 2008; Dortu & Thonart, 2009).

Generally, Lactic acid bacteria are used for this process as inoculum to use against microorganisms to retard their growth and it considered safer because it is already used in human food for years. In the beginning, lactic acid bacteria neglected as inoculum for seafood because normally seafood does not have this bacteria. But if seafood is treated with salt, smoke and with other types of packaging than these bacteria can become more effective, and they will change according to fish and bacterial species change (Leroi, 2010).

Seafood is more susceptible to degrade by microorganisms, to increase the shelf life of such foods there is a method of edible film or coating on food material. These eatable coverings of food are much better than synthetic coverings because these are harmless for human and can easily consume with food (Bourtoom, 2008). These films make a natural layer on a food product to save the food from external environmental factors like control the exchange of gases (oxygen, Carbon dioxide, and ethylene) which are important in respiration process of the food product. It also helps food to stop moisture loss (Embuscado & Huber, 2009). This coating material fully covered the food material and become a permanent part of the food product (Guilbert, 1995). The first edible film used for food safety was made from soymilk in 15th century in japan (Sánchez-Ortega et al., 2014).

In this review, we will discuss different seafood protective techniques like protective cultures and edible films to increase their shelf life.

Fish is very healthy nutritious seafood but it is perishable food so after death its degradation process starts in a short time due to different microorganisms and biochemical reactions (Matak et al., 2015). When we talk about the quality of fish different complicated parameters like nutritional, (Pietrowski et al., 2012) microbiological, metabolically, physicochemical and biochemical are involved. To avoid the loss of this rich nutritional source protective cultures and edible films are a good idea to protect fish from any physical, mechanical and microbiological damage. (Min et al., 2005).

Protective Cultures

Bio-preservation is the most suitable technique used to increase the quality of hygiene and improve microbiological sensory parameters. This technique uses natural microflora and their antimicrobial metabolites (Nilsson et al., 2005; Garcia et al., 2010). The most commonly used microflora is Lactic acid bacteria which produce many types of antimicrobial metabolites like organic acids, acetoin, diacetyl, hydrogen peroxide, antifungal peptides, and bacteriocins. In recent research, there were great advancements to use lactic acid bacteria for biological food preservation (Cleveland et al., 2001; Nilsson et al., 2005; Galvez et al., 2007; Nes, 2011). Seafood texture provides a suitable environment for the production of pathogenic microorganisms (Emborg et al., 2002; Dalgaard et al., 2006). Lactic acid bacteria which are borne by seafood can also be able to grow at refrigerated temperatures and these are completely fit for seafood environment. Their growth can control the potential of pathogenic microorganisms by producing antimicrobial metabolites (Nilsson, 1997; Nilsson et al., 1999; Nilsson et al., 2005; Nes, 2011). Some antifungal compounds also produce from Lactic acid bacteria, so it prevents the growth of a wide range of fungi and used for improvement in sensory characters and microbiological safety of food products (Florou-Paneri et al., 2013).

Genetically modified protective cultures

Some bacterial cultures have very less antimicrobial effects so these are not perfect to use for protective cultures. To solve this problem scientists use genetic engineering for improving the characteristics of already available strains. By using this molecular biology technique some specific genes and enzymes can be targeted and new characteristics can be added by addition or deletion of any specific genes. This idea of genome changes in microorganisms by using genetic engineering was started in Lactic acid bacteria by (McKay & Baldwin, 1990) then yeasts (Chapman, 1991) and fungi (Geisen, 1993).

These changes can be produced by some mutagenic agents or by radiations but this method can also produce some undesirable changes in the genome so other ways of genetic modifications are used for perfect results like genes can be transferred through conjugation process in which plasmids are naturally transferred between cells of closely related species bacteria.

One other method is a transformation in which Pure DNA or genetically modified DNA can be inserting artificially from one species of bacteria to another species of bacteria. So in this process, there is no limitation of related species. These all modifications should be according to the GRAS (Generally recognized as safe) status; either strains of bacteria or their genes all should be safe for food consumers according to food and drug administration (FDA). Those plasmids which are used for the transfer from one species to others should never contain

antibiotic resistance markers or DNA sequence which does not fulfill the requirements of GRAS (generally recognized as safe).

Integrative transformation is a technique through which only genes of interest can be transferred to other bacteria and attached to its chromosome (Berghof & Stahl, 1991). This integration process minimizes the risk of undesirable transfer of genes to other microorganisms because after integration these genes are mostly very stable, so they also overcome the pleiotropic effects of genes. Some starter cultures produce toxic metabolites which have negative effects (Le Bars, 1979). To control this situation gene disruption process can be used through which genes of those useless products can be eliminated from microbial strain (Geisen et al, 1990) or recombinant DNA technology can be used by which these non-desired genes can be inactivated by integration of DNA fragments on the homologous chromosomal site of gene between identical sequences of DNA.

Edible Films

Any light material covering on food is used to increase the shelf life of food product is called coating of edible films. These coatings can be eatable with food or it can be removed from food before eating. Edible films and coatings have the same purpose but the main difference between them like coating is directly applied on food for covering, but the edible film is prepared separately and then applied on food for covering (Cordeiro de Azeredo, 2012).

Edible films should have complete characteristics as its name show like as first part is “edible” it should have all properties of that food on which it applied according to the “Food and Drug administration” because it should be completely safe and healthy for humans (Erkmen & Bozoglu, 2016). The second part is “film” used for covering so it should cover the food material properly that no outer air and moisture disturb the food that it should control the transportation of gas and moisture between food and outer environment. Moreover, it should not have any effect on the physical and mechanical properties of food (Han, 2014; Erkmen & Bozoglu, 2016). Food products quality can be increased by using edible films because they can control the microbiological, oxidative and enzymatic reactions of food and also improve the sensory qualities of food.

Instead of all these qualities, there is a problem that limits the use of edible films to specific applications like their permeability is weaker than synthetic films. Scientists are trying to overcome this problem with further research because customers like natural products more than synthetic products (Kester & Fennema, 1986). Moreover, biodegradability of the edible film is very attractive approach in food industry business because it is cheaper than synthetic packaging material and more attractive for health-conscious customers (Krochta & De Mulder-Johnston, 1997; Arnarante & Banks, 2001).

Characteristics of Edible films

As discussed above the function of edible films to protect food from the interference of external environment and keep food safe and fresh with extending the shelf life. These coverings should be matched with food material compounds to keep it healthy for humans. For this purpose edible films should have the following characteristics:

Edible films should be healthy and approved by food and drug administration and it should be biodegradable after digestion but remain stable physically and mechanically during storage and transportation from one place to another place to avoid external disturbance. This covering should be evenly attached on food surface and with good tensile strength and

permeability of this film should be proper to keep balance environment of air, gases, and moisture for a food product (Guilbert et al., 1996; Bourtoom, 2008; Pavlath & Orts, 2009; Erkmen & Bozoglu, 2016). For keeping all these characteristics maintained in edible films there are some specific components which are mostly used for the formation of film and these all materials are getting from both plants and animals. The detail of these components is given in the Table 1.

Table 1. Components of edible film formation. Source: (Erkmen & Barazi, 2018)

	Food Materials	Examples
Film-forming materials	Animal proteins	Whey protein, collagen, gelatin, casein, egg-white protein, fish myofibrillar protein, feather keratin
	Plant Proteins	Soy protein, corn zein, wheat gluten, pea protein, rice bran protein, cottonseed protein, peanut Protein
	Linear, neutral polysaccharides	Agar, curdlan, cereal b-glucan, methylcellulose, hydroxypropyl methylcellulose, Microcrystalline Cellulose, pullulan, konjac glucomannan, inulin.
	Linear, anionic polysaccharides	Sodium alginate, propylene glycol alginate, carrageenan, pectin, gellan gum, carboxymethylcellulose or cellulose gum
	Linear, cationic polysaccharides	Chitosan
	Linear, substituted, neutral polysaccharides	Fenugreek (<i>Trigonella foenum-graecum</i>), guar gum, tara gum, locust bean gum
	Linear, substituted, anionic polysaccharides	Xanthan gum
	Branched polysaccharides	Gum arabic, gum ghatti, karaya, larch arabinogalactan
	Lipids	Waxes (beeswax, paraffin, carnauba wax, candelilla wax, rice bran wax), acetoglycerides
	Resins	Shellac, terpene, asafoetida, benjoin, chicle, guarana, myrrhe, opoponax, sandarague, styrax
Plasticizers	Polyols	Glycerol, propylene glycol, polypropylene glycol, sorbitol, polyethylene glycol, corn syrup
	Others	Sucrose and water
Additives	Flavors	Oil based flavors, Citrus, Mints, Volatile oils
	Colors	Pigments
	Antimicrobials	Organic acids (acetic, benzoic, lactic, propionic, sorbic); Fatty acid esters (glyceryl monolaurate); Polypeptides (lysozyme, peroxidase, lactoferrin); nitrites and sulfites, chitosan, bacteriocins (nisin, pediocin), parabens, liquid smoke, sodium chloride.
	Antioxidants	Ascorbic acid, 4-hexylresorcinol, amino acids (cysteine and glutathione), citric acid.
	Nutrients	Vitamin E, calcium, zinc, aluminum
	Emulsifiers	Lecithins, mono- and diglycerides, mono- and diglyceride esters, Fatty sucrose esters, fatty alcohols, fatty acids
	Lipid emulsions	Edible waxes, fatty acids
	Probiotic organisms	<i>Bifidobacterium</i> (<i>Bifidobacterium lactis</i> Bb-12)
Plant essential oils	Cinnamon, oregano, lemongrass, savory, sweet inula, vanilin, clove, citronella, thyme	

Active food Packaging

There is an optimistic approach of edible films is to act as a carrier of active substances like antimicrobial compounds that are used to control the biodegradation of food. This method is called active food packaging (Cuq et al., 1995; Han (2000, 2001). Edible films also have an advantage that makes it more suitable than artificial packaging is eatable and biodegradable (Cuq et al, 1995; Han, 2002). These antimicrobial compounds are probiotics, chemical agents and natural extracts.

Antimicrobial compounds should be selected carefully according to the nature of food material and on the basis of the chemical, physical and organoleptic property of active compounds, on the physiology of target microorganisms and on other different regulatory mechanisms (Coma, 2012).

Chemical antimicrobial agents include organic acids and these are produced by different chemical reactions of natural acids (Han, 2005). Mostly used organic acids are acetic acid, lactic acid, sorbic acid, citric acid, and their salts. These acids control microorganisms by reducing the pH, cytoplasm acidification and controlling the permeability of cell membrane (Naidu, 2000). The second category of antimicrobial compounds is natural extracts including essential oils obtained from plants (Burt, 2004). These essential oils are used for food flavor. These have strong aromatic property; to solve this problem can be packed into edible films. Mostly used essential oils are cinnamon, lemongrass, marjoram, clove, ginger, oregano, Eucalyptus globulus, and Ziziphora clinopodioides. These oils are used against many microorganisms due to their phenolic characteristics up to 85% (Burt, 2004; Ahmad et al, 2012; Ejaz et al., 2017; Martucci et al., 2015).

The third category of antimicrobial agents is probiotics and they can stop the growth of other microorganisms. Probiotics are useful to live microorganisms for health (Dehghani et al., 2018) and they include Lactobacillus and Bifidobacterium genus and they can easily compete with harmful microorganisms (Han, 2005). These probiotics also can be packed within edible films due to its efficacy.

Scientific Parameters of Edible Films:

Edible films are important for food safety that's why their composition should be an important concern for food industries. Scientists consider all chemical and physical parameters during the formation of edible films according to specific food components (Han, 2002; Nussinovitch, 2003). Film-forming material should be soluble in water, ethanol, and acetone for wet casting and other properties like glass transition, phase transition, and gelatinization property are important for dry casting (Guilbert et al., 1997). Other properties like viscosity, hydrophilic, hygroscopicity and lipophilic should also be considered for film formation. Those edible films which are formed from protein, polysaccharides, their pH, solubility, salt condition, and denaturation all depend on chemical modifications (Yildirim & Hettiarachchy, 1997; Were et al., 1999). Edible films formation also depends on physical changes like the formation of composites, the addition of particles, lamination, annealing, perforation, over-coating, and heat curing (Gennadios et al., 1996; Miller et al., 1997; Micard et al., 2000).

Edible film formation also depends on mechanical parameters and on the physical chemistry of forming material involves elasticity, strength, the cohesion of polymers, adhesion of film on the surface of food material, light transmittance, gases permeability (Sothornvit & Krochta, 2000). The most important property of edible film should be adhesion (Peyron, 1991; Guilbert et al, 1996) that is an attractive force between the coating film and food product (Anonymous, 1992). So, if adhesion force will be less can't completely cover the food material or can be easily removed from the food material.

CONCLUSION

Seafood is a fully nutritious food with proteins, fats, and vitamins but due to its perishable texture, it leads towards degradation. To solve this problem bioprotective cultures have been developed from starter cultures of microorganisms. These bio-protective cultures produce antimicrobial compounds to kill microbes.

Some protective cultures developed by genetic modification in microbial strains to get desirable functions of microorganisms. Secondly, edible films are used to increase the shelf life of food, improve taste, quality and maintain sensory of food. These films also used for carrying the antimicrobial strains to protect food are called active food packaging. All these food protective techniques should be according to GRAS (Generally recognized as safe) and FDA (Food and drug administration) rules and should be appropriate for consumer need.

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