Antimicrobial films: a review

M. Rocha[1], F. A. Ferreira[1], M. M. Souza[1] and C. Prentice[1,*]

[1]School of Chemistry and Food, Federal University of Rio Grande, Rio Grande RS, Brazil
*Correspondence author: dqmprent@furg.br

Recent foodborne microbial outbreaks are the driving force in the search for innovative ways to inhibit microbial growth in food while maintaining its quality and safety. A new trend in food preservation consists of the incorporation of antimicrobial films on food surfaces. Many studies have demonstrated that antimicrobial films and coatings are effective in reducing levels of pathogenic organisms like *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhi and *Staphylococcus aureus*. Different matrices can be used to incorporate antimicrobial agents, including proteins, lipids, polysaccharides or composites. The most frequent antimicrobials incorporated in food packaging films are organic acid, enzymes, bacteriocins, polysaccharides and essential oils. Peptides are widely recognized as promising alternatives to use as antimicrobials. The aim of the present review is to summarize current information about antimicrobial films and the microorganisms that are inhibited by these antimicrobial agents.

Keywords antimicrobial; films; active packaging.

1. Introduction

Recent foodborne microbial outbreaks are the driving force in the search for innovative ways to inhibit microbial growth in food while maintaining its quality and safety. A new trend in food preservation consists of the use of active packaging in order to enlarge the safety margin and reassure high quality products and the incorporation of antimicrobial agents in films, which can be used as active packaging. Films and coatings with antimicrobial properties have innovated the concept of active packaging, being developed to reduce, inhibit or stop the growth of microorganisms on food surfaces. In most fresh or processed products, the microbial contamination with the highest intensity is found on their surface. Incorporation of antimicrobial compounds into films results in decreased diffusion rates from the packaging material into the product, thus assisting in the maintenance of high concentrations of the active ingredient where it is required. Many studies have demonstrated that antimicrobial agents when incorporated into edible films and coatings could be effective for reducing levels of pathogenic organisms such as *E. coli* O157:H7, *L. monocytogenes, S.* Typhi and *S. aureus*. Different matrices can be used to incorporate antimicrobial agents, including proteins, lipids, polysaccharides or composites. The most frequent antimicrobials incorporated in food packaging films are organic acid (e.g. sorbic, benzoic, citric and propionic acids), enzymes (e.g. lysozyme), bacteriocins (e.g. nisin), polysaccharides (e.g. chitosan) and essential oils (e.g. bergamot, cinnamon, citronella, clove, ginger, oregano, pimento and rosemary). According to [1] peptides are widely recognized as promising alternatives to use as antimicrobial agents. The aim of the present review is to summarize current information about the antimicrobial films and microorganisms that are inhibited by these antimicrobial agents.

According to [2], the protein-based materials experienced a boom of interest in the early twentieth century. Prior to World War II, several types of protein-based films, coatings, plastics and textiles were commercially available. Textiles developed from agricultural proteins were categorized as azlons. World War I and World War II created a substantial demand for materials to make uniforms, blankets and other supplies for soldiers. Wool was the main textile material, but wool substitutes were also created from such products as casein, soy, corn zein and peanut protein [3]. Casein-based protein fibers had silk-like properties, and were commercialized under brand names such as Lanital, Merinova and Arlac developed by the United States Department of Agriculture (USDA). Peanut protein fiber was sold as Ardil, and corn zein was used to make the commercialized textile Vicara [4].

The inherent properties of proteins make them excellent starting materials for films and coatings. The distribution of charged, polar and non-polar amino acids along the protein chain creates chemical potential. For example, in beta-lactoglobulin, the major protein found in whey, the shading illustrates the domains of polar and non-polar areas along the protein chain. The resulting interactive forces produce a cohesive protein film matrix. Films are formed and stabilized through electrostatic interactions, hydrogen bonding, Van der Waals forces, covalent bonding, and disulfide bridges [5]. Protein film-forming capabilities are best demonstrated in emulsified systems in which amphipathic proteins form films at air–water or water–oil interfaces. There are also secondary benefits for using proteins to form films and coatings. Proteins have multiple sites for chemical interaction as a function of their diverse amino acid functional groups, which can allow for property improvement and tailoring. Chemical changes can improve the stability of films and coatings. Cross-linked protein films are often more stable than their polysaccharide-based counterparts and have a longer shelf life [2,6].

In general the protein-based films and coatings are also biodegradable and compostable. As they degrade, they provide a source of nitrogen, which contributes a fertilizer benefit not available from other non-protein-based films and

coatings. Finally, there is emerging evidence that the bioactive peptides produced upon digestion of proteins (dairy sources in particular) have antihypertensive and radical scavenging health benefits [7, 8]. Many antimicrobial peptides (AMPs) have potent activity against bacteria, including those that are resistant to conventional antibiotics. Their activity is often relatively-specifically directed against certain genera or groups of bacteria, which could limit damaging effects on a patient's commensal flora. AMPs generally exhibit high stability to wide ranges in pH and temperature, characteristics that may be beneficial for their scaled-up production and formulation into deliverable products. Moreover, due to their rather specific modes of action, many AMPs exhibit low toxicity for eukaryotic cells, providing an alternative of use. Their typical mode of action is also linked to a low propensity for resistance development in target bacteria. These unique features differentiate AMPs from many conventional antibiotics [9].

2. Methods of preparation of antimicrobial films

Elaboration of films has been possible thanks to the film forming capacity of natural polymers. Such ability is related to the chemical structure of these compounds, which allows the association through hydrogen bonding of their polymeric chains [10]. Many studies show that the most frequently used macromolecule (polymers) for antimicrobial films formulation are proteins (whey protein, wheat gluten protein, soy protein, triticale protein, pea protein, fish protein), polysaccharides (chitosan, starch, hydroxypropylmethylcellulose, carboxymethylcellulose) and blends of both [11-16]. The preparation of antimicrobial films must include at least one component capable of forming a suitable continuous, cohesive and adhesive matrix. Such a formulation comprises a film-forming agent (macromolecule), solvent (water, ethanol and others), plasticizers (glycerol, sorbitol and others), pH adjusting agent (acid, sodium hydroxide and others) when necessary, and an antimicrobial agent [13-14,16]. Subsequently, the film-forming solution is poured on a support and submitted to a dehydration process that allows an easy release from the mold or applied in a coating product [17].

As cited by [18], the film formation may involve the following mechanisms: melting and solidification, used on hard fats and waxes; simple coacervation, which consists in the precipitation of a hydrocolloid dispersed in this aqueous solution. This precipitation can be obtained by solvent evaporation (drying), by adding a non-electrolyte solute, and wherein the hydrocolloid is insoluble (e.g. ethanol) by an electrolyte which induces precipitation or intersection components, or modifying the pH of the solution; complex coacervation, which consists in obtaining the precipitation by mixing two hydrocolloid solutions with opposite electric charges that interact to form the complex polymer; Gelation or thermal coagulation, which consists in heating the macromolecules involving denaturation , gelling and precipitation. Antimicrobial films can be formed in different ways, which may affect their properties via several processes depending on the starting material [19].

The composition of the films contains a substance that forms a tough and solid matrix, through the interactions between the molecules undergo chemical or physical treatments. The methods that can be used in the preparation of antimicrobial films include: casting, tape-casting, extrusion and thermopressing. The casting method is one of the most used methods by researchers in the preparation of films and comprehends the preparation of a colloidal solution of the macromolecule (polymers) added or not of additives, dispersion of solution in adequate support and, drying under strictly controlled conditions. After evaporation of the solvent, the dried film is removed from the support [20].

Antimicrobial films were prepared by incorporating different levels of sodium lactate (NaL) and ε-polylysine (ε-PL) into sorbitol-plasticized whey protein isolate (WPI) films [21]. Whey protein isolate and glycerol were mixed to form a matrix to incorporate antimicrobial agents and produce edible films with antimicrobial activity against *Listeria monocytogenes* strains isolated from cheeses [22]. Antimicrobial biodegradable films have been prepared with sweet potato starch by incorporating potassium sorbate or chitosan [23]. The antimicrobial activities against *E. coli* O157:H7, as well as the stability of carvacrol, the main constituent of oregano oil, were evaluated during the preparation and storage of apple-based edible films made by casting method [24]. This method of preparation of films has been used extensively in research on films but has some disadvantages such as the difficulty of incorporating materials of different nature, problems of withdrawal of the support film used for the casting and difficulty in scaling (scale-up to industrial scale)[20].

One technique still slightly known to the preparation of hygroscopic films is the tape-casting or spread-casting. The technique consists in controlled spreading of a suspension on a surface to be dried, which can be an alternative for the preparation of films with dimensions much greater than those of films prepared by traditional casting. The thickness of the suspension is controlled by means of a blade (doctor-blade) attached to the lower part of the spreading device and drying the film is held on the support itself, whose temperature can be controlled and on which they can circulate heated air. This method can be applied to the production of polysaccharides and proteins films in general. The production of composite-films (based on cassava starch, glycerol, and cellulose or sisal fibers) was performed by the tape-casting technique, aiming at the preparation of biodegradable bags with different thickness, by [24].

The extrusion method is used for obtaining films to be adapted for industrial scale production, with the following advantages: it is fast, takes up less space, has a lower cost of production and the process is already employed in the production of conventional synthetic packaging. The extrusion process is continuous, where the sample is melted, molded and dried leaving the extruder. Basically, it consists of the entry of the polymer into a cylinder heated by the action of one or two rotary screws, this material is previously softened and forced into a matrix to obtain continuous

forms. The extruder performs heat exchange between the walls of the cylinder, the screw and the material. It also behaves as a chemical reactor, since it is capable of cooking, denature and sterilize using high temperature and pressure. In their study [26], developed active biodegradable packaging, able to increase the shelf-life of minimally processed fruit. This was obtained by extrusion and thermopressing of a mass of starch gel (starch suspended in water) and additives (plasticizers and / or active agents).

In the process of thermopressing, the filmogenic mixture is placed below a mold heated by electrical resistors placed inside the equipment. The upper part is then placed down, so that the mass is spread over the surface of the mold. Upon heating, the water begins to evaporate from the mixture and, the vapor causes expansion of the dough filling the whole mold, causing solidification of the material which can be removed when still warm from the machine.

3. Antimicrobial films

Active compounds, such as antimicrobials, can be incorporated into biodegradable films and can act as active packing. This alternative is developed to reduce, inhibit or stop the growth of foodborne pathogens and others microorganisms on food surfaces [27]. In processed or fresh products, the microbial contamination is found with the highest intensity on their surface requiring a system to control the growth of microorganisms. The direct addition of antimicrobial agents in foods can inhibit their antimicrobial effect, because different components of these foods can decrease their efficiency. The implementation of films as active packaging can be more efficient than antimicrobial additives used in the foods, since they can migrate selectively and gradually from active film compounds to the surface of the food [28]. The antimicrobial films are divided into two groups: in the first, the agent migrates to the surface of product, while in the second they are effective against microbial growth surface without the need for migration into the product. In these cases, an intense contact between the product and the antimicrobial agent [29].

The selection of an antimicrobial agent depends on its activity against a target microorganism. The growth of potential microorganisms that can spoil food is predictable due to the food product characteristics such as pH, water activity, composition, as well as storage conditions. The direct incorporation of additives in packaging films is a convenient methodology by which antimicrobial activity can be achieved [30]. The incorporation of active agents into polymeric systems results in a variety of release profiles with different stages. In some cases, the additive release has been described as a simple matrix diffusion process, with degradation occurring at a later stage, post active substance release [31]. Furthermore, the diffusion of antimicrobials from the film depends on the size, shape (linear, branched, or cyclic), and polarity of the diffusing molecule, as well as the chemical structure of the film and the degree of molecular crosslinking. The different interactions between the polymer matrix and antimicrobial agent can lead to various degrees of inhibition against pathogens [32]. It should be noted that the antimicrobial activity also depends on the type of the active compounds, its concentration and microorganism specie.

Besides the potential use of antimicrobial peptides as alternatives to antibiotics with respect to the treatment/prevention of disease, numerous recent reports have also described the activity of antimicrobial peptides against bacteria growing in biofilms, that is, communities encased in structured matrices of extracellular macromolecules, on medical devices. Medical devices can become colonized with bacteria growing in biofilms, which are resistant to elements of the host immune response and to extremely high concentrations of conventional antibiotics. Consequently, resolution of medical device-related infections usually requires the removal of the device. Notably, low concentrations of some AMPs have been shown to have inhibitory and disruptive properties that can eliminate even well-established biofilms [9].

In general, some cell characteristics can suggest possible sites of action of an antimicrobial agent. A microbial cell contains enzymes responsible for many metabolic processes; a semipermeable membrane (the cytoplasmic membrane) which maintains the integrity of the cellular contents, selectively controlling the transport of substances between the cell and its surroundings, in addition to being the site of some enzymatic reactions. The cell wall provides protection to the bacteria, participating in certain physiological processes. Damage to any of these levels can initiate changes that lead to cell death [33].

There are several categories of antimicrobials that can be potentially incorporated into films as showed in Table 1.

Table 1 Antimicrobial used in films which showed antimicrobial activity against some microorganisms

Matrix film	Antimicrobial agent	Microorganisms	Reference
Gelatin from skin	bergamot and lemongrass essential oils	*E. coli, L. monocytogenes, S. aureus* and *S.* Typhimurium	[34]
Whey protein	potassium sorbate	*E. coli* O157:H7	[35]
Whey protein	malic acid, nisin and natamycin	*Penicillium aeruginosa, Y. lipolytica, L.monocytogenes , Penicillium commune* and *Penicillium chrysogenum*	[22]
Hydroxypropylmethylcellulose	kiam wood (*Cotyleobium lanceotatum*) extract	*E. coli* O175:H7, *S. aureus* and *L. monocytogenes*	[12]
Carboxymethylcellulose	potassium sorbate	*Aspergillus flavus*, *Aspergillus parasiticus*	[36]
Whey protein	lactic and propionic acids, chitooligosaccharides and natamycin	*E. coli, S. aureus* and *Y. lipolytica*	[14]
Soy protein	grape seed extract, nisin, and EDTA	*E. coli* O175:H7, *L. monocytogenes* and *S.* Typhimurium	[16]
Wheat gluten	potassium sorbate	*Aspergillus niger* and *Fusarium incarnatum*	[37]
Whey protein	p-aminobenzoic and sorbic acids	*L. monocytogenes*, *E. coli* O157:H7, and *S. typhimurium*	[32]
Fish skin gelatin and egg white	clove essential oil	*C. perfringens, E. faecium, P. aeruginosa, V. parahaemolyticus* and *Y. enterocolitica*	[38]
Chitosan-gelatin	chitosan	*E. coli* and *L. monocytogenes*	[13]
Pea protein isolate, hydroxypropylmethylcellulose, methylcellulose or sodium caseinate	bacteriocins	*L. innocua*	[15]
Triticale protein	oregano essential oil	*S. aureus, E. coli* and *P. aeruginosa*	[39]
Chitosan	propionic acid	*S. aureus*, *Salmonella* spp. *Candida* spp. and *Penicillium* spp.	[30]
Chitosan	chitosan	*E. coli* and *L. plantarum*	[40]
Sunflower protein	clove essential oils	*B. cereus*, *Clostridium perfringens*, *P. fluorescens*, *V. parahaemolyticus*, *B. coagulans*, *E. faecium*, *A. niger* and *P. expansum*	[41]

Essential oils are categorised as GRAS (generally recognised as safe) by U.S. Food and Drug Administration [42]. Many essential oils have been used as antimicrobial into films including bergamot (*Citrus bergamia*), cinnamon (*Cinnamomum verum*), citronella (*Pelargonium citrosum*), coriander (*Coriandrum sativum*), clove (*Syzygium aromaticum* L.), cypress (*Cupressus sempervirens* L.) fennel (*Foeniculum vulgare* Miller*),* garlic (*Allium sativum*), ginger (*Zingiber officinale*), lavender (*Lavandula stoechas*), lemongrass (*Cymbopogon citratus*), oregano (*Origanum vulgare*), pine (*Pinus sylvestris*), rosemary (*Rosemarinus officinalis*), sage (*Salvia officinalis*) and thyme (*Thymus*

vulgaris) [21, 34, 38-39, 41, 43, 45-46]. The antimicrobial activity of essential oils is assigned to a number of small terpenoids and phenolic compounds like thymol, carvacrol and eugenol [34, 37]. Terpenes have the ability to disrupt and penetrate into lipid structure of the bacteria cell wall, leading to denaturation of proteins and destruction of cell membrane, cytoplasmic leakage, cell lysis and eventually cell death [43]. The decrease in pH that occurs due to cell membrane disruption resulted in a loss of control of cellular processes such as DNA transcription, protein synthesis and enzyme activity [47]. The effect of antimicrobial films incorporated with essential oil was verified against many pathogens like *E. coli*, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus, S.* Enteritidis and *S.* Typhimurium [34, 38, 41, 48].

Chitosan is a polysaccharide that can be derived from deacetylation of chitin, a biopolymer that is abundant in a variety of crustacean shells, such as crab shells, fungi cell walls and other biological materials, having antimicrobial properties against some bacteria, fungi and yeast [49-50]. The potential of chitosan to act as an antimicrobial agent on films against microrganisms as *E. coli*, *S. aureus*, *L. monocytogenes*, *B. cereus*, *S.* Typhimurium, *Zygosaccharomyces bailii*, *L. plantarum, Penicillium, Aspergillus, Rhizopus* and others was reported for many studies [13, 40, 51-53]. Chitosan has significant antimicrobial activity owing to its NH_2 group. It can be protonated to NH_3^+ and readily forms electrostatic interactions with anionic groups of microbial cell membranes, leading to the leakage of proteinaceous fluid causing disruption on the cell [40, 52].

Chitooligosaccharide (COS) is the oligosaccharide fraction prepared by chemical or enzymatic hydrolysis of chitosan. It is known to possess antifungal and antibacterial activity [14, 54]. The aforementioned properties occur due to the positively charged chitooligosaccharide molecules, which interact with negatively charged bacteria causing disruption on the cell [54]. In many studies, films incorporated with chitooligosaccharide have antimicrobial activity against *S. aureus*, *E. coli*, *Y. lipolytica* [14].

Organic acids and their salts have a long history as GRAS (generally recognized as safe) food preservatives by U.S. Food and Drug Administration. A lot of research has been performed using films as retention matrices of preservatives' agents such as, potassium sorbate, sorbic acid, p-aminobenzoic, malic acid, lactic acid, propionic acid and others in matrix different (protein and polysaccharides). These organic acids can be efficient against pathogenic bacteria such as *E. coli* O157:H7, *L. monocytogenes*, *S.* Typhimurium, *S. aureus*, *B. cereus* and on some fungi like *A. niger*, *A. flavus*, *A. parasiticus* and *F. incarnatum* [14, 22, 30, 32, 35-37, 52]. Basically, the antimicrobial activity of organic acids occurs due to pH reduction, disruption of substrate transport, and reduction of proton motive force [33]. As protonated acid diffuses across the microbial membrane, an alkaline environment is encountered, favoring the dissociation of the acid into the acid anion and free proton. In addition to shifting the internal pH out of range for optimal enzymatic activity, protein and DNA/RNA synthesis are adversely affected by the presence of organic acids at elevated levels [14, 33, 36].

Nisin is used in the food industry as a safe and natural preservative and has been studied for its suitability to be incorporated into whey protein isolate, soy protein isolate, chitosan and others films [16, 22, 52]. Nisin is an antimicrobial peptide (bacteriocin) produced by strains of *Lactococus lactis* subsp. *lactis*, that exhibits antimicrobial activity towards a wide range of Gram-positive bacteria but shows little or no activity against Gram-negative bacteria [52, 55-57]. Its mode of action includes the inhibition of cell wall synthesis and the formation of pores in the cytoplasmic membrane [10]. The film incorporated with nisin can be efficient against pathogenic bacteria such *L. monocytogenes*, *S. aureus* and *B. cereus* [16, 52].

Compounds from plant extract, such as grape seed extract, showed effective inhibitory activities against foodborne pathogens [47]. Inhibition of pathogens by the natural extracts occurs due to the hydroxyl groups and conjugated double bonds in the reactive groups [16]. The groups of chemicals present in plant extracts include polyphenols, quinones, flavanols/flavanoids and others. Soy protein films incorporated with grape seed extract demonstrated the greatest inhibitory activity against *L. monocytogenes* [14]. The antimicrobial properties of hydroxypropylmethylcellulose films containing kiam wood extract showed inhibitory activity against *L. monocytogenes*, *S. aureus* and *E. coli* [12].

4. Methods used to evaluate antimicrobial activity in edible films

Numerous studies have been performed to establish the effectiveness of antimicrobials in films. The election of the method depends on the purpose of the assay, the nature of the antimicrobial and the characteristics of target microorganisms, among others [10]. According to Clinical and Laboratory Standards Institute (CLSI) [58], the developing disk diffusion tests consist in inoculum preparation, inoculation of test plates, disk application on inoculated agar plates, incubation at specific conditions and measure of inhibition zone surrounding of the disk [10, 44, 58]. Generally, the inoculant should contain a turbidity standard corresponding to the number 0.5 of the McFarland scale, which represents a total of 1 - 2 x 10^8 CFU/mL. The zones of film inhibition discs on the plates can be examined by measuring their diameter and by visual observations through photographs at different times of inhibition, the data was expressed in surface units (mm), subtracting the film area from the inhibition zone [14, 30]. The enumeration by plate count of microbial population, at selected times, from inoculated surface agar plates in contact with film disk containing the antimicrobial is a useful test to model wrapping of foods and obtained results that may suggest what can happen when the film enters in contact with a contaminated surface [10, 59].

Antimicrobial activity by diffusion of antimicrobials from the film disk depends on the size, shape, and polarity of the diffusing molecule, as well as the chemical structure of the film [32]. This test gives information of the ability of the antimicrobial incorporated in the film to inhibit microbial growth at a prefixed time [10]. This method can be used for different microbial strains including: *L. acidophilus*, *S. cholerasuis*, *L. innocua*, *Citrobacter freundii*, *E. coli*, *Shigella sonnei, P. aeruginosa, Y. enterocolitica, Brochothrix thermosphacta, S. aureus, B. cereus, L. monocytogenes, C. perfringens*, *Aeromonas hydrophila, Photobacterium phosphoreum, Shewanella putrefaciens, P. fluorescens, Vibrio parahaemolyticus, B. coagulans, Bifidobacterium animalis subespecies lactis, Bifidobacterium bifidum, Enterococcus faecium, Lactobacillus helveticus, Debaryomyces hansenii, A. niger, P. expansum,* among others [41, 59-62].

[40] used two methods to evaluate the antimicrobial activity. On one hand, agar diffusion method adapted by [13] was performed. Agar plates were spread with 0.1 mL of inoculum containing approximately 10^5–10^6 CFU/mL of bacteria. Films (sterilized with UV light) were cut into a disc shape of 15 mm diameter and then placed on the surface of MRS (Man Rogosa and Sharpe) agar for *L. plantarum* strain and on LB (Lysogeny Broth) agar for *E. coli*. After incubation for 24 h at 37 °C for E. coli and at 28 °C for *L. plantarum* strains, the plates were optically examined for width of inhibition in the contact area. The total area was used to evaluate the antimicrobial potential of films. In the case of film forming solutions, 30 µL of solution were poured into agar wells and the same procedure described above was applied. On the other hand, the antimicrobial effect of chitosan films and film forming solutions in the bacterial growth were also evaluated by the suspension culture medium assay on 96-well polypropylene microplates. Cell cultures were incubated at 28 °C for *L. plantarum* CECT748 and at 37 °C for *E. coli* 0157:H7 for 24 h in both cases. The liquid culture assay was conducted in 200 µL of both broths with film pieces (sterilized with UV light) or 60 µL of film forming solutions. Each well was inoculated with 2% (v/v) of an overnight bacteria culture. Antimicrobial activity by diffusion of antimicrobials from the film disk depends on the size, shape, and polarity of the diffusing molecule, as well as the chemical structure of the film [32]. This test is an end point assay and gives information of the ability of the antimicrobial incorporated in the film to inhibit microbial growth at a prefixed time [10].

[63] evaluated the antimicrobial activity by zone inhibition method with a modified agar. Films based on kudzu starch–chitosan were cut in 6 mm diameter disks then placed on nutrient agar in Petri dishes that had been seeded with 20 µl of bacterial cell suspensions. The Petri dishes were examined for zone of inhibition after 24 h incubation at 37 °C. The results showed that the kudzu starch–chitosan films inhibited the growth of the bacteria *E. coli* and *S. aureus*. [64] studied film performance to prevent microbial contamination of a high water activity (aW) product. Sabouraud agar with aw depressed to 0.98 by the addition of dextrose and the pH adjusted to 4.5 with 50% (w/w) citric acid solution were formulated to resemble that kind of products. The inoculum of *Lactobacillus* spp. and *Zygosaccharomyces bailii* (NRRL 7256) was prepared in Sabouraud broth and incubated until the early stationary phase was achieved (24 h). To carry out the assay, the *Lactobacillus* spp. inoculum was added to temperate Sabouraud agar and after that, was poured into 9 cm diameter plates. The plates were examined for clear zones around the wells (inhibition zones) after 24 h. The zone diameters were measured in triplicate and the means were reported.

Chitosan films incorporated with propionic acid were assessed for the antimicrobial capacity of the active packaging by using the agar diffusion method. The film disks (1 and 3 cm in diameter) were placed by pressing them to ensure contact with the agar surface innoculated with suspension of 10^7CFU/ ml of *Salmonella* spp. and *S. aureus*. This microorganisms were inhibited by the film studied [30].

Films based on whey protein isolate containing sodium lactate (NaL) and ε-polylysine (ε -PL) utilized such active packaging on fresh beef. In this packaging, the counts of microrganisms such as the total viable count, Lactic Acid Bacteria and *Pseudomonas* spp. were evaluated. The use of films containing antimicrobial resulted in a significant reduction of the total flora and pseudomonads population, total viable count and Lactic Acid Bacteria were reduced when compared with films without sodium lactate (NaL) and ε -polylysine (ε -PL) [21].

Effects of soy protein edible films containing oregano or thyme essential oils were tested on fresh ground beef during refrigerated storage (at 4 °C). Films applied on ground beef patties reduced counts of coliform and *Pseudomonas* spp. [43].

The enumeration by plate count of microbial population, at selected times, from inoculated surface agar plates in contact with film disk containing the antimicrobial is a useful test to model wrapping of foods and obtained results may suggest what can happen when the film enters in contact with a contaminated surface [10, 59]. The film surface inoculation test is another frequently performed assay and consists in the enumeration by plate count of the microbial population inoculated on the surface of a film disk in contact with a semisolid media such as agar that model a certain food product [10]. This assay is useful to simulate surface contamination. Results obtained may suggest what happens when microbial contamination occurs on coatings or films in contact with food and gives an idea of the barrier ability of the film to prevent an external contamination [65-66].

5. Application of antimicrobial films for foods

Use of packaging films containing antimicrobial agents could be more than the direct addition of these compounds onto the food. Antimicrobial packaging is a promising and rapidly emerging technology in which antimicrobial agents are incorporated into or coated onto food packaging materials to prolong the shelf-life of the packed food. According to

[67], the simple categorization of microorganisms may be very helpful to select specific antimicrobial agents. Such categories may consist of oxygen requirement (aerobes and anaerobes), cell wall composition (Gram-positive and Gram-negative), growth-stage (spores and vegetative cells), optimal growth temperature (thermophilic, mesophilic and psychrotropic) and acid/osmosis resistance. Researchers such as [67], doing an overall comparison of the efficacy of different antimicrobials, say that in the packaging films, combinations of antimicrobials provided better efficacy, and it is likely that research will move in the direction of finding cost effective and highly active combinations of antimicrobials. The inclusion of several antimicrobials possibly adds complexity and cost to the manufacturing process of films, but in cases where it is feasible to replace some of an expensive antimicrobial with a cheaper one and still achieve higher antimicrobial activity with this combination, costs for the film could possibly even decrease. Even when antimicrobial films fail to completely remove higher numbers of unwanted microbes, they can act as an additional, post-processing safety measure. Several antimicrobial packaging films should be able to provide antimicrobial activity even during or after such processing steps as heat or pressure treatments, and can exert antimicrobial activity on the few remaining microbes. It can also be imagined that antimicrobial films could allow processing to be done at lower temperatures or pressures, thus reducing processing costs without compromising food safety. In the end, the acceptance of particular antimicrobial films by the food industry will probably depend on the regulatory climate and the balance between the cost of the antimicrobial film and the benefit of a second antimicrobial hurdle. However, the use of proper packaging technology to minimize food losses and provide safe and sound food products has always been the focus of food packaging [69]. Nanotechnology has potential to greatly influence the packaging sector. Nanoscale innovations in the forms of pathogen detection, active packaging, antimicrobial packaging and barrier formation are poised to elevate food packaging to new heights.

References

[1] Marcos, J F, Gandia, M. Antimicrobial peptides: to membranes and beyond. *Exp. Op. on Drug Discovery*. 2009;4: 659-671.

[2] Dangaran, K, Tomasula, PM, Qi, P. *Structure and function of protein-based edible films and coatings*. In: Embuscado, ME, Huber, KC. *Edible films and coatings for food applications*. Springer, Dordrecht Heidelberg , London. 2009, 403.

[3] Brooks, M. Forgotten fibres: issues in the collecting and conservation of regenerated protein fibres. In: Rogerson C, Garside P. The Future of the Twentieth Century: Collecting, Interpreting and Conserving Modern Materials, London , Archetype , 2006, 33 – 40.

[4] Kiplinger, J. *Meet the Azlons from A to Z*: Regenerated & Rejuvenated. Fabrics.net January/ February. 2003.

[5] Krochta JM , Baldwin EA , Nisperos-Carriedo, MO. *Edible Coatings and Films to Improve Food Quality* . CRC Press LLC, Boca Raton, FL. 1994.

[6] Barone JR , Schmidt WF. Nonfood applications of proteinaceous renewable materials. *Journal of Chemical Education*. 2006; 83: 1003 – 1009.

[7] Aimutis WR. Bioactive properties of milk proteins with particular focus on anticariogenesis. *The Journal of Nutrition*. 2004; 134: 989S – 995S.

[8] FitzGerald, RJ, Murray, BA , Walsh DJ. Hypotensive peptides from milk proteins. *The Journal of Nutrition*. 2004; 134: 980S – 988S

[9] Upton, M, Cotter, P, Tagg, J. Antimicrobial peptides as therapeutic agents. International Journal of Microbiology. 2012; 1-2.

[10] Campos, CA, Gerschenson, LN, Flores, SK. Developm ent of Edible Fil ms and Coatings with Antimicrobial Activity. *Food Bioprocess Technol*. 2011; 4: 849– 875.

[11] Bourtoom T. Review article: Protein edible film: Properties enhancement. *International Food Research*, 2009; 16:1- 9.

[12] Chana-Thaworn, J, Chanthachum, S, Wittaya, T. Properties and antimicrobial activity of edible films incorporated with kiam wood (*Cotyleobium lanceotatum*) extract. *LWT - Food Science and Technology*. 2011; 44: 284-292.

[13] Pareda, M, Ponce, AG, Marcovich, NE, Ruseckaite, RA, Martucci, JF. Chitosan-gelatin composites and bi-layer films with potential antimicrobial activity. *Food Hydrocolloids*. 2011; 25: 1372-1381.

[14] Ramos, OL, Silva, SI, Soares, JC, Fernandes, JC, Poças, MF, Pintado, ME, Malcata, FX.. Features and performance of edible films, obtained from whey protein isolate formulated with antimicrobial compounds. *Food Research International*. 2012; 45: 351–361.

[15] Sánchez-González, L, Saavedra, JIQ, Chiralt, A. Physical properties and antilisterial activity of bioactive edible films containing *Lactobacillus plantarum*. *Food Hydrocolloids*. 2013 ; 33: 92- 98.

[16] Sivarooban, T, Hettiarachchy, NS, Johnson, M.G. Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films. *Food Research International*. 2008 ; 41:781–785.

[17] Cuq, B, AYMARD, C, CUQ, JL, GUILBERT, S. Edible packaging films based on fish myofibrillar proteins: Formulation and functional properties. *Journal of Food Science*. 1995. 60: 1369–1374.

[18] Quintavalla, S, Vicini, L. Antimicrobial food packaging in meat industry. *Meat Science*. 2002;62:.373–380.

[19] Milda EE, Kerry CH. *Edible Films and Coatings for Food Applications*. Springer, Science Business Media, LLC, New York, 2009.

[20] Mali S, Debiagi F, Grossmann MVE, Yamashita F. Starch, sugarcane bagasse fibre, and polyvinyl alcohol effects on extruded foam properties: A mixture design approach. *Industrial Crops and Products*. 2010; 32: 353–359.

[21] Zinoviadou K, Koutsoumanis KP, Biliaderis C G. Physical and thermomechanical properties of whey protein isolate films containing antimicrobials, and their effect against spoilage flora of fresh beef. *Food Hydrocolloids*. 2010; 24: 49–59.

[22] Pintado CMBS, Ferreira MASS, Sousa I. Properties of Whey Protein-Based Films Containing Organic Acids and Nisin to Control Listeria monocytogenes. *Journal of Food Protection*. 2009; 72: 1891-1896.

[23] Xiao LS, Jia MW, Yonghong C, Guohua Z. Antimicrobial and physical properties of sweet potato starch films incorporated with potassium sorbate or chitosan. *Food Hydrocolloids*. 2010;. 24: 285–290.
[24] Du WX, Olsen CW, Avena-Bustillos RJ, Mchugh TH, Levin CE, Friedman M. Storage stability and antibacterial activity against *Escherichia coli* O157:H7 of carvacrol in edible apple films made by two different casting methods. *Journal of Agricultural and Food Chemistry*. 2008; 56: 3082–3088.
[25] Yang, WS, Biamino, S, Padovano, E, Pavese, M, Marchisio, S, D'Amico, G, Mio, SC, Chen, PF, Badini, C.Thermophysical properties of SiC multilayer prepared by tape casting and pressureless sintering.*Composite Structures*. 2013; 96: 469–475.
[26] Cerisuelo, JP, Bermúdez, JM, Aucejo, S, Catalá, R, Gavara, R, Hernández-Muñoz, P. Describing and modeling the release of an antimicrobial agent from an active PP/EVOH/PP package for salmon. *Journal of Food Engineering*. 2013; 116: 352-361.
[27] Appendini, P, Hotchkiss, JH. Review of Antimicrobial Food Packaging. *Innovative Food Science & Emerging Technologies*. 2002; 3: 113.
[28] Falguera, V, Quintero, JP, Jiménez, A., Muñoz, JA, Ibarz, A. Edible films and coatings: Structures, active functions and trends in their use. *Trends in Food Science & Technology*. 2011; 22: 292-303.
[29] Vermeiren, l, Devlieghere, F, Debevere, J. Effectiveness of some recent antimicrobial packaging concepts. *Food Additives and Contaminants*. 2002;19:163-171.
[30] Rivero, S, Giannuzzi, MA, Pinotti, A. Controlled delivery of propionic acid from chitosan films for pastry dough conservation. *Journal of Food Engineering*. 2013; 116:524–531.
[31] Gallagher, KM, Corrigan, OI. Mechanistic aspects of the release of levamisole hydrochloride from biodegradable polymers. *Journal of Controlled Release*. 2000; 69: 261–272.
[32] Cagri, A, Ustunol, Z, Ryser, ET. Antimicrobial, mechanical, and moisture barrier properties of low pH whey protein-based edible films containing p-aminobenzoic or sorbic acids. *Food Microbiology and Safety*. 2001; 66: 865-870.
[33] Mani-López, E, García, HS, López-Malo, A. Organic acids as antimicrobials to control Salmonella in meat and poultry products. *Food Research International*. 2012; 45: 713-721.
[34] Ahmad, M, Benjakul, S, Prodpran, T, Agustini, TW. Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. *Food Hydrocolloids*. 2012; 28: 189-199.
[35] Pérez, LMA, Balagué, CE, Rubioloc, AC, Verdinia, RA. Evaluation of the biocide properties of whey-protein edible films with potassium sorbate to control non-O157 shiga toxin producing *Escherichia coli*. *Procedia Food Science*. 2011; 1: 203-209.
[36] Sayanjali, S, Ghanbarzadeh, B, Ghiassifar, S. Evaluation of antimicrobial and physical properties of edible film based on carboxymethyl cellulose containing potassium sorbate on some mycotoxigenic *Aspergillus* species in fresh pistachios. *LWT - Food Science and Technology*. 2011 ; 44 : 1133-1138.
[37] Türe, H, Gallstedt, M, Hedenqvist, MK. Antimicrobial compression-moulded wheat gluten films containing potassium sorbate. *Food Research International*. 2012 ; 45 :109–115.
[38] Giménez, B, Gómez-Guillén, MC, López-Caballero, ME, Gómez-Estaca, J, Montero, P.Role of sepiolite in the release of active compo unds from gelatin e egg white films. *Food Hydrocolloids*. 2012 ; 27: 475- 486.
[39] Aguirre, A, Borneo, R, León, AE. Antimicrobial, mechanical and barr ier properties of triticale protein films incorporated with oregano essential oil. *Food Bioscience*. 2013; 1 : 2-9.
[40] Leceta, I, Gurrero, P, Ibarburu, I, Dueñas, MT, Caba, K. Characterization and antimicrobial analysis of chitosan-based films. *Journal of Food Engineering*. 2013; 116: 889–899.
[41] Salgado, PR, López-Caballero, E, Gómez-Guillén, MC, Mauri, AN, Montero, MP. Sunflower protein films incorporated with clove essential oil have potential application for the preservation of fish patties. *Food Hydrocolloids*. 2013; 33: 74-84.
[42] Persico, P, Ambrogi, V, Carfagna, C, Cerruti, P, Ferrocino, I, Mauriello, G. Nanocomposite polymer films containing carvacrol for antimicrobial active packaging. *Polymer Engineering & Science*. 2009; 49: 1447-1455.
[43] Emirŏglu, KZ, Yemis, GP, Coskun, BK, Candogan, K. Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. *Meat Science*. 2010 ; 86: 283–288.
[44] Gómez-Estaca, J, Lacey, LA, López-Caballero, ME,Gómez-Guillén, MC, Montero, P.Biodegradable gelatin–chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. *Food Microbiology*. 2010; 27: 889-896.
[45] Pires, C, Ramos, C, Teixeira, B, Batista, I, Nunes, ML, Marques, A . Hake proteins edible fi lms incorporated with essential oils: physical, mechanical, antioxidant and antibacterial properties. *Food Hydrocolloids*, 2013; 30 : 224-231.
[46] Tongnuanchan, P, Benjakul, S, Prodpran, T. Properties and antioxidant activity of fish skin gelatin film incorporated with citrus essential oils. *Food Chemistry*. 2012; 134: 1571-1579.
[47] Oussalah, M, Caillet, S, Salmieri, S, Saucier, L, Lacroix, M. Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. *Journal of Agricultural and Food Chemistry*. 2004; 54: 5598–5605.
[48] Rojas-Graü, MA, Avena-Bustillos, RJ, Olsen, C, Friedman, M, Henika, PR, Martín-Belloso, O, Pan, Z, McHugh, TH.Effects of plant essential oils and oil compounds on mechanical, barrier and antimicrobial properties of alginate–apple puree edible films. *Journal of Food Engineering*. 2007; 81: 634–641.
[49] Coma, V, Martial-Gros, A, Garreau, S, Copinet, F, Salin, F, Deschamps, A. Edible antimicrobial fi lms based on chitosan matrix. *Journal of Food Science*. 2002 ; 67: 11 62-1169.
[50] Kim, KW, Min, BJ, Kim, YT, Kimmel, RM, Cooksey, K, Park, SI. Antimicrobial activity against foodborne pathogens of chitosan biopolymer films of different molecular weights. *LWT - Food Science and Technology*. 2011 ; 44 : 565- 569.
[51] Li, LH, Deng, JC, Deng, HD, Liu, ZL, Li, XL.Preparation, characterization and antimicrobial activities of chitosan/Ag/ZnO blend films. *Chemical Engineering Journal*. 2010; 160: 378–382.
[52] Pranoto, Y, Raksh it, SK, Salokhe, VM. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *LW T –Food Science and Technology*. 2005; 38: 859–865.
[53] Rao, MS, Kanatt, SR, Chawla, SP, Sharma, A. Chitosan and guar gum composite films: Preparation, physical, mechanical and antimicrobial properties. *Carbohydrate Polymers*. 2010; 82:1243–1247

[54] Fernandes, JC, Tavaria, FK, Soares, JC, Ramos, OS, Monteiro, J, Pintado, ME, Malcata, FX.Antimicrobial effects of chitosans and chitooligosaccharides, upon *Staphylococcus aureus* and *Escherichia coli*, in food model systems. *Food Microbiology*. 2008; 25: 922-928.
[55] Branen, JK., Davidson, PM. Enhancement of nisin, lysozyme, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin. *International Journal Food Microbiology*. 2004; 90: 63–74.
[56] Malheiros, PS, Daroit, DJ, Silveira, NP, Brandelli, A.Effect of nanovesicle-enc apsulated nisin on growth of Listeria monocytogenes in milk. *Food Microbiology*. 2010; 27:175–178.
[57] Taylor, TM, Bruce, BD, Weiss, J., Davidson, PM. *Listeria monocytogenes* and *Escherichia coli* O157:H7 inhibition in vitro by liposome-encapsulated nisin and ethylene diaminetetraacetic acid. *Journal Food Safety*. 2008; 28: 183–197.
[58] CLSI- *Clinical laboratory standards institute*. Document M2-A8 - Performance Standards for Antimicrobial Disk Susceptibility Test; Approved Standard – Eight Edition.23, 1, 2003.
[59] Kristo, E, Koutsoumanis, KP, Biliaderis, CG. Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on *Listeria monocytogenes*. *Food Hydrocolloids*. 2008; 22: 373–386.
[60] Broumand, A, Emam-Djomeh, Z, Hamedi, M, Razavi, SH.Antimicrobial, water vapour permeability, mechanical and thermal properties of casein based Zataraia multiflora Boiss. Extract containing film. *LW T - Food Science and Technology*. 2011; 44: 2316-2323.
[61] Manab, A, Sawitri, ME, Alawwaly, KU, Purnomo, H. Antimicrobial activity of whey protein based edible film incorporated with organic acids. *African Journal of Food Science*. 2011; 5: 6–11.
[62] Zivanovic, S, Chi, S, Draughon, AF. Antimicrobial activity of chitosan films enriched with essential oils. *Journal of Food Science*. 2005; 70: 45-50.
[63] Zhong, Y, Song, X, Li, Y. Antimicrobial, physical and mechanical properties of kudzu starch–chitosan composite films as a function of acid solvent types. *Carbohydrate Polymers*. 2011; 84: 335–342.
[64] Vásconez, MB, Flores, SK, Campos, CA, Alvarado, J, Gerschenson, LN. Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings. *Food Research International*. 2009; 42: 762–769.
[65] Flores, S, Fama , L, Rojas, AM, Goyanes, S, Gerschenson, L. Physical properties of tapioca-starch edible films: Influence of film making and potassium sorbate. *Food Research International*. 2007; 40: 257–265.
[66] Wang, S, Duan, H, Zhang, W, LI, JW. Analysis of bacterial foodborne disease outbreaks in China between 1994 and 2005. *FEMS- Immunology and Medical Microbiology*. 2007; 51:8-13.
[67] La Storia, A. *Development and application of antimicrobial food packaging*. Ph D Thesis. Università degli Studi di Napoli Federico II, Napoli, Italy, 2012.
[68] Joerger, RD. Antimicrobial films for food applications: a quantitative analysis of their effectiveness. *Packaging Technology and Science.* 2007; 20: 231–273.
[69] Emamifar, A. Applications of antimicrobial polymer nanocomposites in food packaging, Advances in nanocomposite technology, Dr. Abbass Hashim (Ed.), ISBN: 978-953-307-347-7. Available at: http://www.intechopen.com/books/advances-in-nanocomposite-technology/applications-of-antimicrobial polymer- nanocomposites-in-food-packaging. Accessed May, 1, 2013.

