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Antimicrobial Efficiency of Edible Films in Food Industry

Dan Cristian VODNAR[1], Oana Lelia POP[1], Francisc Vasile DULF[2], Carmen SOCACIU[1]*

[1] *University of Agricultural Sciences and Veterinary Medicine, Department of Food Science, 3-5 Manastur St., 400372 Cluj-Napoca Romania; dan.vodnar@usamvcluj.ro; oana.pop@usamvcluj.ro; carmen.socaciu@usamvcluj.ro (*corresponding author)*

[2] *University of Agricultural Sciences and Veterinary Medicine, Department Environmental and Plant Protection, 3-5 Manastur St., 400372 Cluj-Napoca, Romania; francisc.dulf@usamvcluj.ro*

Abstract

In the current article, several applications of materials in food packaging and food safety are reviewed, including: polymers as high barrier packaging materials, natural substances as potent antimicrobial agents, and the efficiency of antimicrobial films in food industry. The first condition of all this materials in order to be utilised in food industry is to fall into the list of generally accepted as food grade. Active antimicrobial food packaging systems are supposed not only to passively protect food products against environmental factors, but also to inhibit or retard microbial growth on the food surface, extending the shelf life of products. Edible films can be incorporated into conventional food packaging systems with a dual purpose as an edible and antimicrobial component. Several benefits resulting from the application of the antimicrobial films, embedded with various natural antimicrobials, on different food products as fruits, vegetables and meat products lead to various benefits as maintaining the products freshness for longer time, extend product shelf life and reduce the risk of pathogen growth. Thus, food graded antimicrobial packaging films are promising food packaging materials because its biodegradability provides sustainable development for modern community by reducing the use of drugs as chemical antibiotics, reducing economic losses and overall increases the trust of the final consumer in food products.

Keywords: antimicrobials, edible films, fruits and vegetables, meat products, natural compounds

Introduction

The current global market has experienced an increasing of the consumers demand for better quality of life. Meeting the criteria of high quality and reduce the use of chemicals in order to obtain food safety products are few of the factors contributing to this demand. This has lead companies and researchers to explore different ways to improve their productivity in terms of maintaining quality, freshness and food safety, such as using sustainable materials in food packaging (Mahalik and Nambiar, 2010). The emergence of bacterial antibiotic resistance and negative consumer attitudes regarding chemical food preservatives has induced an increased interest in the use of natural antimicrobial molecules as alternative agents for the control of food spoilage and hazardous pathogens. Foodborne diseases produce a wide range of diseases and are activated by agents that were ingested along with food. As declared by the authorities [World Health Organization (WHO), Centres for Disease Control and Prevention (CDC)], the spoilage of food may occur at any stage in the process from food production to consumption ("farm to fork") and in many of this cases the pathogens are to blame (Srey *et al.*, 2013).

There has been an increasing interest during recent years to develop materials with film-forming capacity and having antimicrobial properties that help to improve health safety and shelf life (Fakhouri *et al.*, 2015; Genskowsky *et al.*, 2015).

Antimicrobial packaging is one of the most effective in killing or inhibiting pathogenic microorganism growth that contaminate foods (Salleh *et al.*, 2007). Contamination could occur when food is being exposed under distinct conditions during slaughtering, post processing, distribution and shipping. (Sung *et al.*, 2013). Thus, a good packaging is required in order to reduce the contamination into the food.

There is a current trend in food packaging systems concerning about developing new innovative concepts to inhibit pathogenic microbial activities in food (Ge *et al.*, 2015; Ollé Resa *et al.*, 2014). Up to now, products as active and intelligent packaging systems have been developed in order to meet significant safety requirements (Han, 2014). Antimicrobial packaging located within the group of active packaging are made from wrapping system containing antimicrobials (natural or synthetic agents) (Sung *et al.*, 2013). Utilization of antimicrobial packaging is more advantages compare to direct adding of antimicrobial agents onto food products due to the fact that the cover, containing the active agent, can be remove just before product consumption so the antimicrobial will not be ingested together with the product (Salgado *et al.*, 2015). Surface application of antimicrobials represents an alternative to direct application and is based on the incorporation of an antimicrobial compound into an edible coating that is then applied on the food surface (Vodnar, 2012). The choice of active agents that may be

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incorporated into edible films could influence the consumers perception regarding the ingredients listed on product label (Vodnar, 2012).

Edible films are thin films prepared from materials, which act as a barrier to external factors and thus protect the food product, extend its shelf life and improve its quality (Suyatma *et al.,* 2005). An edible film is defined as a performed thin layer or solid sheets of material placed on or between food components (Galus and Kadzińska, 2015). The edible films could be applied on food products as film wraps or pouches for food. The use of edible films in food protection and preservation has increased in the last decade since they can offer several advantages (high antimicrobial effect with minimum sight effects, increasing the trust of the consumer, reducing the economic costs etc.) over synthetic materials, such as being biodegradable and environmentally friendly (Tharanathan, 2003).

The most commonly occurring and applied natural polymers include: polysaccharides (starch, cellulose and its derivatives, alginate, chitosan, gellan gum), proteins (collagen, zein, soybean, gluten proteins, milk proteins) and fats (beeswax, candelilla wax, fatty acids and glycerols) (Casariego *et al.,* 2008). One important advantage of this type of packages is the fact that they might be consumed together with the food product (Suyatma *et al.,* 2005).

The functionality of edible film materials may vary, as each component confers different properties on the composite matrix. Polysaccharides or proteins used in film formulation usually have suitable mechanical and gas barrier properties and poor water vapour barrier properties. On the other hand, films from lipids exhibit good water vapour barrier properties, poor mechanical strength, and high oxygen permeability.

Antimicrobial food packaging materials

Antimicrobial packaging can be generally divided into two major groups: biodegradable packaging and non-biodegradable packaging. The synthetic polymers have the advantages of low cost materials, low density, inert, excellent barrier properties, good mechanical strength, high transparency, ability to be heat sealed and easy to be printed, but are not biodegradable (Akter *et al.,* 2012; Bonilla *et al.,* 2012; Pattanayaiying *et al.,* 2015). The most common plastics used in food packaging are: low, linear and high density polyethylene, polypropylene, polystyrene, ethylene vinyl acetate, polyethylene terephtalate and polyvinyl chloride (Sung *et al.,* 2013). The main disadvantages of these materials are: the diffusion of additives from polymers into food products and the negative impact to the environment (Akter *et al.,* 2012).

Currently, biodegradable antimicrobial films are produce from natural polymers incorporating natural or synthetic antimicrobial agents (Han, 2014). Most of the literature reports are focused on the blending of thermoplastic starches with biodegradable polyesters such as: polycaprolactone, polylactic acid, polyhydroxibutyrate-co-hydroxyvalerate, polybutylene succinate-adipate, poly (butylenes adipate-co-therephtalate) and poly (hydroxyl ester ether) (Agarwal, 2012; Mahieu *et al.,* 2015; Terzopoulou *et al.,* 2015).

Polylactic acid (PLA)

Is one of the most promising biopolymer (due to relative low melting temperature, its ability to form compact films, increase the biocompatibility of many active accents etc.) with industrial applications able to replace the petroleum-derived polymers. PLA is linear aliphatic thermoplastic polyester derived from 100% renewable resources, which is produced by the fermentation of biomass (Armentano *et al.,* 2013; Vodnar *et al.,* 2010). It is a thermoplastic polymer with high-strength, high-modulus, good processability, being completely biodegradable, compostable, and biocompatible (Djidi *et al.,* 2015; Ingrao *et al.,* 2015; Jašo *et al.,* 2015). In addition, PLA is safe for the food packaging application since it has been proven GRAS for use in food packaging to fulfill the requirements for direct contact with aqueous, acidic and fatty foods (Rhim, 2013). PLA's potential for consumer products such as packaging is remarkable due to its transparency, low toxicity and environmentally benign characteristics (Dutta *et al.,* 2009). There are limited studies investigating the antimicrobial efficiency of antimicrobials as lemon extract, thymol, lysozyme (Del Nobile *et al.,* 2009), olive leaf extract (Marcos *et al.,* 2014) incorporated into PLA films.

Chitosan

A linear polysaccharide consisting of (1,4)-linked 2-amino-deoxy-β-D-glucan, is a deacetylated derivate of chitin which is the second most abundant polysaccharide found in nature after cellulose (Dutta *et al.,* 2009). The deacetilation is usually incomplete, thus chitosan is a copolymer comprised of deacetylated and acetylated units. Chitosan has been found to be non-toxic, biodegradable, biofunctional, biocompatible in addition to heaving antimicrobial characteristics (Jayakumar *et al.,* 2007). Chitosan is especially of interest for the antimicrobial packaging and biomaterials industry because is cationic and naturally antimicrobial properties. Comparing with other bio-based food packaging materials, chitosan has the advantage of being able to incorporate active and functional substances with antimicrobial activities against *Listeria monocytogenes* for example (Moller *et al.,* 2004; Vodnar, 2012).

Chitosan because of the positive charge has a better antimicrobial activity than chitin. The exact mechanism of chitosan and its derivatives is still perfectly unknown, but the literature reported different potential explanations (Rabea *et al.,* 2003). One of the reasons of the antimicrobial character of chitosan is its positively charged amino group, which interacts with negatively charged microbial cell membranes (Shahidi *et al.,* 1999). On the other hand, chitosan acts as chelating agent that selectively binds trace metals and thus inhibits the production of toxins and microbial growth (Cuero *et al.,* 1991). The antimicrobial mechanism of chitosan were different from Gram-positive (*S. aureus*) and Gram negative (*E. coli*) bacteria (Zheng and Zhu, 2003). The authors have reported the effects of chitosan on *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria. The antimicrobial activity increased once with the molecular weight of chitosan against *S. aureus*, while for *E. coli*, the antimicrobial activity increased once with decreasing molecular weight of chitosan. The authors proposed one explanation for each antimicrobial activity: (I) in case of *S. aureus*, chitosan form a polymer membrane, which inhibits nutrients from entering the cell and (II) for *E. coli*, chitosan entered the cell through pervasion.

Chitosan nanoparticles are prepared by interaction of oppositely charge macromolecules (de Azeredo, 2013). Chitin whiskers (crystalline nanofibrils) can be prepared by acid hydrolysis of chitin and a subsequent diacetylation of chitin whiskers produces chitosan whiskers. Chitosan nanoparticles and their derivatives have wide antimicrobial effects (Madureira *et al.*, 2015). Chitosan nanoparticles (in concentrations from 0,1 to 0,7% w/v) present higher antimicrobial effect than bulk chitosan because of their higher surface area and charge density, providing a higher interaction with the cationic surface of bacterial cells (Madureira *et al.*, 2015).

The antimicrobial effect of nanostructures combining chitosan and other antimicrobial agents has been studied by many researchers (Ramani *et al.,* 2014; Song and Jang, 2014). Carvacrol–loaded chitosan nanoparticles were obtained by oil-in-water emulsion followed by ionic gelation of chitosan (Keawchaoon and Yoksan, 2011). The minimum inhibitory concentration indicated that carvacrol increased the antimicrobial activity of chitosan nanoparticles against *E. coli*, *S. aureus*, *Bacillus cereus*.

Pectin

Is one of the main components of the plant cell wall, contributing to tissue rigidity and integrity and is considered one of the most complex macromolecules in nature (Bayarri *et al.,* 2014; Benito-Peña *et al.,* 2016; De'Nobili *et al.,* 2015; Tripathi *et al.,* 2010). The most important sources for pectin extraction are apple pomace and citrus peels (Videcoq *et al.,* 2011). Pectin is an ingredient used in food industry with no limitation other than current good manufacturing practice, is considered as generally recognized as safe by FDA, and it was used in food mainly as gelling, stabilizing or thickening agent in products such as yogurts, jams, drinks and ice cream (Laurent and Boulenguer, 2003).

Soy protein isolate

Is a complex mixture of proteins with widely different molecular properties; ranging from 200 to 600 kDa. The major soybean protein group is globulins, which can be fractionated in 2 S, 7 S, 11 S and 15 S according to their sedimentation coefficient. The 7 S and 11 S fractions are the main fractions and represent between 31% and 37% of the total extractable protein. This fractions have the capability of polymerization (Cho and Rhee, 2004). Soy protein isolate is a protein with reproducible resource, good biocompatibility, biodegradability, processability and film forming capacity that has significant potential for use in the food industry, agriculture, bioscience and biotechnology (Khan *et al.,* 2012).

Alginates

Are structural polysaccharides extracted from brown algae. In molecular terms, alginates are linear water-soluble polysaccharides comprising (1-4) linked units of R-D-mannuronate and *â*-L-guluronate at different proportions and different distributions in the chain (Hambleton *et al.,* 2011). Most applications of alginate is based on its gel-forming ability through cations binding; the transition from water-soluble sodium alginate to water insoluble calcium alginate (Galus and Kadzińska, 2015). Sodium alginate-based edible films can be used to limit dehydration of meat, fish and fruits. Alginate can also be support of active substances, like encapsulation agent (Pop *et al.,* 2015).

Whey proteins

Have exceptional nutritional value and functional (Banerjee and Chen, 1995). More, liquid whey is produced in large quantities and its annual production increases continuously (Banerjee and Chen, 1995). The establishment of edible films and coatings from whey proteins can increase the utilization of whey, improve nutritional value of foods and prolong the shelf life (Ozdemir and Floros, 2008). Whey protein isolates represent the purer form of whey proteins and have shown promising mechanical features. Other valuable properties of the whey protein isolates are moderate moisture permeability (McHugh *et al.,* 1994) and good oxygen barrier properties-comparable to those exhibited by the best synthetic polymer based films available.

Natural compounds in antimicrobial edible films

The antimicrobial substances most frequently introduced into films are: bactericins, enzymes, oils, plant extracts and preservatives (Angiolillo *et al.,* 2014; Calo *et al.,* 2015; Ríos and Recio, 2005; Wang et *al.,* 2015). The antimicrobial activities of essential oils and plant extracts are well known for a long time and numerous research papers (Acevedo-Fani *et al.,* 2015; Akhavan *et al.,* 2015; Ankri and Mirelman, 1999; Aumeeruddy-Elalfi *et al.,* 2015; Basgedik *et al.,* 2015; Cottigli *et al.,* 2001; Pesavento *et al.,* 2015) have been published on the antimicrobial activities of plant essential oils against foodborne pathogens. Essential oils are complex mixtures of volatile organic compounds produced as secondary metabolites in plants; they are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, aldehydes, ketones, lactones, phenols and phenol esters) and possess highly inhibitory potential to a wide spectrum of microorganisms. A number of authors have mentioned the antimicrobial activity of essential oils; however, the mechanism of action has not been studied in detail (Kon and Rai, 2013). It is generally believed that essential oils principally performed against the cell cytoplasmic membrane of microorganism (Zabka *et al.,* 2014). The essential oils hydrophobicity enables them to be accumulated in cell membranes, disturbing the structures and causing an increase of permeability. Leakage of intracellular components and impairment of microbial enzymes systems can than occur (Carson *et al.,* 2002) and extensive loss of the cell contents will cause the cell death (Lv *et al.,* 2011). Examples of plant extracts and essential oils most widely incorporated into food packaging systems are: linalool, thymol, carvacrol, clove oil, cinnamaldehyde basil essential oils and teas (Mihaly Cozmuta *et al., 2015*).

Due to the consumers demand with less use of chemicals on minimally processed food products, more attention has been paid in searching for naturally occurring substances able to act as alternative antimicrobials and antioxidants (Atarés *et al.,* 2010).

Some studies have shown that essential oils of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), cinnamon (*Cinnamon cassia*), lemongrass (Cymbopogon *citrates*) and clove (*Eugenia caryphyllata*) are among the most active against strain *E. coli* (Emiroğlu *et al.,* 2010; Martucci *et al.,* 2015; Pranoto *et al.,* 2005b; Zivanovic *et al.,* 2005). The effectiveness of these compounds has been reported previously, more carvacrol (the major component of oregano and thyme) appears to receive the most attention from researchers.

Friedman *et al.* (*2002*), investigated 120 naturally plant derived oils and oil compounds for their antimicrobial activities against four species of foodborne pathogens. The most active oils in terms of BA_{50}values (% of oil in phosphate buffer that killed 50% of bacteria) for *Campylobacter jejuni* (BA_{50}, 0.003-0.009%) are: marigold, ginger root, jasmine, patchouli, gardenia, cedarwood, carrot seed, celery seed, mugwort, spikenard and orange bitter; and for *E. coli* O157:H7 (BA_{50}, 0.046-0.14%): oregano, thyme, cinnamon, palmarosa, bay leaf, clove bud, lemon grass and allspice, for *Listeria monocytogenes* (BA_{50}, 0.057-0.092%): gardenia, cedarwood, bay leaf, clove bud, oregano, cinnamon, allspice, thyme and patchouli, for *Salmonella enteric* (BA_{50}, 0.045-0.14%): thyme, oregano, cinnamon, clove bud, allspice, bay leaf, palmarosa and marjoram.

More, Friedman *et al.* (2004) showed that carvacrol, oregano and cinnamaldehyde were effective antibacterial against antibiotic resistant *B. cereus*, *C. jejuni*, *E .coli*, *S. enteric* and *S. aureus*. Films with preservatives additions such as benzoates, sorbates should be characterized by a wide range of activities against pathogenic microorganisms which can cause food spoilage (Quintavalla and Vicini, 2002).

The most popular products of microbial metabolism are: nisin-a bacteriocin of *Lactoobacillus lactis* bacteria and pediocin -a bacteriocin of *Pedicococcus acidilactici* bacteria (Tong *et al.,* 2014) (Table 1). Literature reports have been describing attempts of their application in many matrices. Nisin is usually applied in film based on proteins of whey, zein, wheat and soybean (Ko *et al.,* 2001). A significant antimicrobial effect against *Salmonella enteritidis* and *Listeria monocytogenes* was achieved in case of film based on zein with nisin addition (Hoffman *et al.,* 2001).

Coma *et al.* (*2001*) described the antimicrobial activity of nisin incorporated into cellulose or hydroxypropylmethylcellulose film against *L. innocua* and *S. aureus*. Other investigations were conducted with nisin being into a film from methylcellulose and hydroxypropylmethylcellulose witch are good matrices for bactericidal substances immobilization and are characterized by elasticity and resistance. The obtained films showed an inhibitory activity of *Micrococcus luteus* growth. Another investigated bacteriocin was pediocin which was introduced into the cellulose film and demonstrated the inhibitory effect (Ming *et al.,* 1997) on the growth of *L. monocytogenes* in concentrations of 7.75 μg/cm^2. More promising results were achieved in a study conducted with pullulan film enriched with sakacin A. The results demonstrated a significant effect, at concentrations of just 1 mg/cm^2, of the film on the reduction of *L. monocytogenes* bacteria (Trinetta *et al.,* 2010).

Enzymes of animal origin include lysozyme, protein isolated from hen egg and milk enzyme: lactoferrin and lactoperoxidase. The immobilization of lysosyme into the whey protein film exerted an inhibition on the growth of *L. monocytogenes* (Min *et al.,* 2008) (Table 1). Lysosyme was added into a film from zein in combination with EDTA, enhancing the lysosyme effect against *E. coli* (Güçbilmez *et al.,* 2007). More, the utilization of lysosyme in combination with nisin and EDTA on alginate film showed strong properties, inhibiting the growth of *M. luteus*, *L. innocua*, *S. enteritidis*, *E. coli* and *S. aureus*. So far, literature references have been describing the feasibility of introducing lactoferrin and lactoperoxidase to a film from whey proteins. The results demonstrated that only the film with the addition of lactoperoxidase displayed the activity against *L. monocytogenes*, *E. coli*, *Salmonella enterica* and *Penicillium commune* (Min and Krochta, 2005).

Sivarooban *et al.* (2008) investigated the effect of grape seeds extract introduced into soybean film (Table 1). The grape seeds extract reduced the growth of *L. monocytogenes* with 1 logarithmic unit, *E. coli* with 0.1 logarithmic units and *S. typhimurium* with 0.2 logarithmic units. More, the combination of grape seeds extracts with EDTA and nisin significantly enhanced the antimicrobial activity of soybean films. (Kanmani and Rhim, 2014) reported that grapefruit extract was successively introduced into carrageenan film and was effective against *M. luteus*, *L. innocua*, *S. enteritidis*, *E. coli*, *S. aureus*.

Antimicrobials preservatives used in edible films are benzoic acid, sodium benzoate, sorbic acid and potassium sorbate (Table 1). These compounds incorporated into edible films have been tested against a wide range of microorganisms. Methylcellulose and chitosan film containing 2% and 4% of sodium benzoate of potassium sorbate inhibited the growth of *Penicillium notatum* and *Rhodotorula ruba* (Chen *et al.,* 1996).

Corn zein film with the addition of sorbic acid was shown to inhibit the growth of *L. monocytogenes* on sweet corn (Carlin *et al.,* 2001). More, the sorbic acid incorporated into whey protein inhibited the growth of *L. monocytogenes*, *E. coli*, *S. typhimurium* on sliced bologna and summer sausage (Cagri *et al.,* 2001; Cagri *et al.,* 2002). The film with potassium sorbate reduced significantly the number of *E. coli* (Shen *et al.,* 2010)

Food applications of antimicrobial edible films

Fruits and vegetables

Concerning nutritional aspects, fresh fruits and vegetables, stored under appropriate conditions are a veritable source of vitamins and minerals, bringing benefits to all systems in the body. A current problem with a large resonance for these products consists in the microbial load on their surface (Aponiene *et al.,* 2015; Oliveira *et al.,* 2015). Fruits and vegetables resistance to various microorganisms is closely correlated with a number of features: anatomical structure of the products, protective substances such as waxes, antimicrobial substances: organic acids, essential oils-feticide substances, formation of chemical compounds in place where the infection occurred (Năsui *et al.*, 2013).

Currently, application of films to fruits and vegetables have received reasonable attention because can serve as carriers in order to maintain fresh quality, to extend product shelf life and to reduce the risk of pathogen growth (Cerqueira *et al.,* 2009; Galus and Kadzińska, 2015; Fakhouri *et al.,* 2015). The most important commercial properties of fruits and vegetables include colour, texture, appearance, nutritional value, flavour and microbial safety (Lin and Zhao, 2007). After harvest, the vegetal products need special conditions to reduce the biological reactions, which accelerate the natural loss of fruit tissue, water loss, change appearance and texture decreasing the commercial value of the product (Nelson, 2015; von Germeten and Hirsch, 2015).

Edible film utilization on fresh fruits and vegetables would: provide a moisture barrier on the product surface in order to decrease the moisture loss; provide a sufficient gas barrier to control gas exchange between the fresh product and its surrounding atmosphere, in an effort to slow respiration, delay

Table 1. Antimicrobials incorporated in edible polymer film

Antimicrobial	Polymer	Targeted microorganism	References
Oregano oil	Soy protein	*E. coli* O157:H7 *S. aureus* *E. coli*	Emiroğlu *et al.* (2010)
	Whey protein	*E. coli* O157:H7 *S. aureus* *S. enteritidis* *L. monocytogenes* *L. plantarum*	Seydim and Sarikus (2006)
Garlic oil	Alginate	*S. aureus* *B. subtilis* *E. coli O157:H7* *E. coli* *S. typhimurium* *S. aureus* *B. cereus*	Liakos *et al.* (2014); Pranoto *et al.* (2005a)
Grape seed extract	Soy protein	*Listeria monocytogenes* *E. coli* *S. typhimurium*	Sivarooban *et al.* (2008)
Grapefruit seed extract	Carrageenan	*Micrococcus luteus* *L. innocua* *S. enteritides* *E. coli* *S. aureus*	Kanmani and Rhim (2014)
Green and black teas extract	Chitosan	*L. monocytogenes*	Vodnar (2012)
Lysozyme	Whey protein	*L. monocytogenes*	Min *et al.* (2008)
	Zein	*L. plantarum* *B. subtilis*	Mecitoğlu *et al.* (2006)
Lactoperoxidase	Whey protein	*L. monocytogenes* *E. coli* *S. enteric* *P. commune*	Min and Krochta (2005)
Nisin	Sodium caseinate	*L. monocytogenes*	Cao-Hoang *et al.* (2010)
Pediocin	Cellulose	*L. monocytogenes*	Ming *et al.* (1997)
Sakacin A	Pullulan	*L. monocytogenes*	Trinetta *et al.* (2010)
Potassium sorbate	Starch	*E. coli*	Shen *et al.* (2010)

deterioration and protect the fresh produce from brown discoloration and texture softening during storage; disable the exchange of volatile compounds between product and environment by providing gas barriers; protect from physical damage of produce caused by mechanical impact, pressure, vibrations and other mechanical factors; and act as carriers for other functional ingredients (Lin and Zhao, 2007).

Năsui *et al.* (2013) evaluated the antimicrobial effect of wash edible films containing green tea extract on red pepper, tomato, apple and nectarine. The authors found that pepper has the highest initial microbial load, followed by nectarine, apple and tomato. After fruits or vegetable washing, a slight decrease in the number of germs is happening for all mentioned products. Fruits and vegetables, which have applied label, showed a considerable decrease in the total number of germs. Edible films made from chitosan and green tea proved effectiveness in all samples, in particular on pepper, where the authors reported the absence of microorganisms on the peel after washing while on tomato were registered germs at 1 CFU/cm^2, on apple 3 CFU/cm^2 and on nectarine 99 CFU/cm^2 (Fig. 1).

Meat Products

According to Juneja *et al.* (2006) and Friedman *et al.* (2009) when carvacrol was incorporated into apple films both *Clostridium perfringens* and *E. coli* O157:H7 decreased dramatically in cooked ground beef and hamburger beef patties.

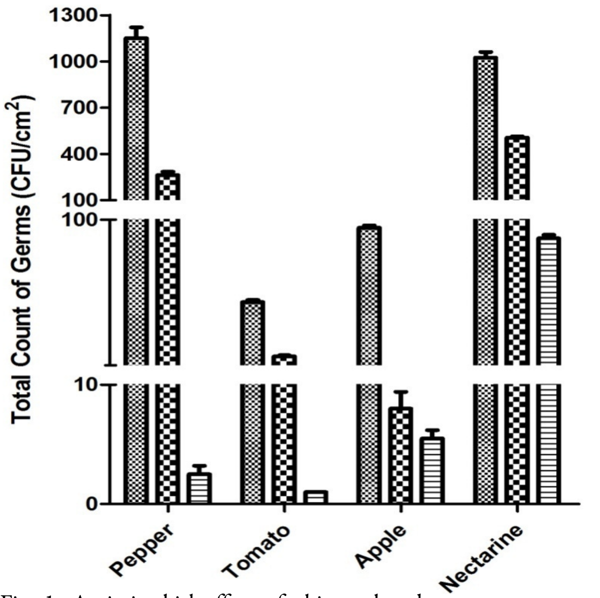

Fig. 1. Antimicrobial effect of chitosan-based green tea extracts ▩ initial number of germs on fruits and vegetable surface; ▦ fruits and vegetables conventionally washed; ▤ fruits and vegetables washed with chitosan films. The antimicrobial effect quantified by the total number of germs (cfu/cm^2) (Năsui *et al.*, 2013)

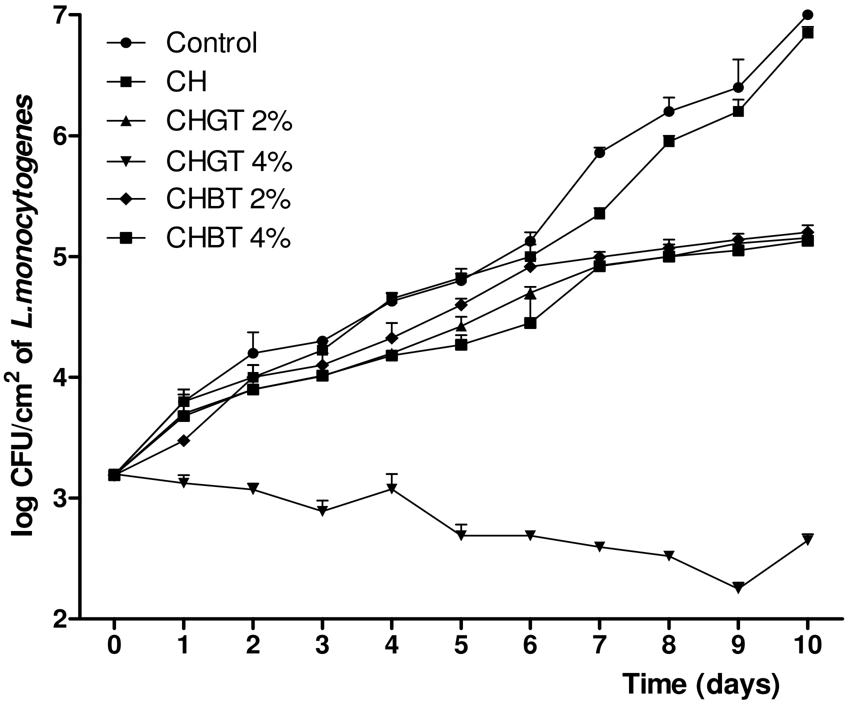

Fig. 2. Antimicrobial effect of chitosan-coated plastic films (■) incorporating 2% (▲), 4% (▾) Green tea and 2% (♦), 4% (▪) Black tea extracts on the growth of *L. monocytogenes* on ham steak at room temperature storage *versus* control sample (●) (Vodnar, 2012).

Emiroğlu *et al.* (2010) obtained a soy protein film incorporated with oregano, thyme that did not have significant effects on total viable counts. More, the authors reported when lactic acid bacteria and *Staphylococcus ssp.* were applied on ground beef patties significantly reductions in coliforms and *Pseudomonas ssp.* counts were observed. When chitosan-based antimicrobial films containing cinnamaldehyde were used on drier surfaces of meat (bologna), *Enterobacteriacease* and S. *liquefaciens* growth were delayed or completely inhibited (Ouattara *et al.,* 2000).

The antimicrobial milk-protein-based film containing essential oils from oregano and pimento were used against *E. coli* O157:H7 and *Pseudomonas ssp.* in preserving whole beef muscle (Oussalah *et al.,* 2004). Incorporating oregano extract into film showed more effective antimicrobial ability, achieving reductions of around 1 log unit for each bacterial species at the end of storage compared with uncoated samples.

Previous study reported by Vodnar (2012) combined the properties of a chitosan film with green and black tea extracts. Incorporation of tea extracts into the chitosan films considerably enhanced the effectiveness against *L. monocytogenes* ATCC 19115 on ham steak.

Green tea (4%) incorporated into chitosan-coated plastic film had a better antilisterial effect than green tea 2% or black tea 2% and 4%. Data from study provided new establishment options for developing antimicrobial packaging films using tea extracts in order to improve the microbiological safety and quality of ham steak during room and refrigerated storage (Vodnar, 2012).

The initial concentration of *L. monocytogenes* ATCC 19115 on inoculated ham steak samples was 3.2 log CFU/cm^2 (Fig. 2). *L. monocytogenes ATCC 19115* in chitosan-coated plastic films grew to 6.8 log CFU/cm^2 after 10 days of storage at room temperature. Incorporating natural extracts into chitosan showed that the growth of *L. monocytogenes* ATCC 19115 was inhibited. The efficacy of antimicrobial effect of tea extracts incorporated into chitosan-coated plastic film was dose dependent. The 2% green tea and 2%, 4% black tea extracts had inhibitory effect on *L. monocytogenes* ATCC 19115 but the most efficient antimicrobial effect has been registered by 4% green tea.

Inoculated ham steak samples with an initial population of 3.2 log CFU/cm^2, were stored at 4°C for 8 weeks. Counts of *L. monocytogenes* ATCC 19115 on ham steak samples treated with chitosan-coated film incorporating tea extracts are shown in Fig.3. In the first 4 weeks, *L. monocytogenes* ATCC 19115 in control and chitosan-coated plastic film grew very slowly from 3.2 to 4.3 log CFU/cm^2. The tea extracts addition into chitosan-coated films were effective against *L monocytogenes* ATCC 19115 and reduced its counts from 3.2 to 1–1.5 log CFU/cm^2 during 8 weeks of storage. In general, the gram negative bacteria are more resistant to polyphenols than Gram positive bacteria, perhaps due to the different cell wall compositions (Mocan *et al.,* 2014; Negi *et al.,* 2003).

Gómes-Estaca *et al.* (2010) reported that gelatin-chitosan film incorporating clove essential oils reduced drastically the population of gram-negative bacteria on

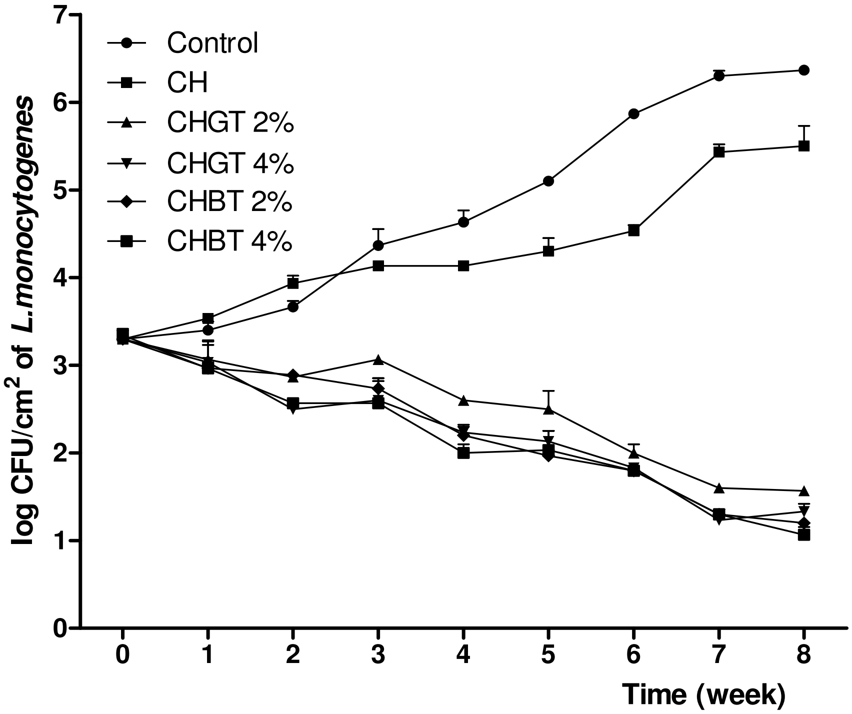

Fig. 3. Antimicrobial effect of chitosan-coated plastic films (■) incorporating 2% (▲), 4% (▾) Green tea and 2% (♦), 4% (▪) Black tea extracts on the growth of *L. monocytogenes* on ham steak store at 4 °C *versus* control sample (●) (Vodnar, 2012)

codfish during chilled storage. More, Gómes-Estaca *et al.* (2007) reported that the stability of cold-smoked sardine muscle was improved by coating the muscle with functional gelatine film.

Conclusions

Antimicrobial packaging is very promising system for the future improvement in food quality and preservation during processing and storage. Natural antimicrobials as one of the food preservative ingredient are expected to substitute synthetic additives for fulfilling the demand of the consumers on safer food additives. The functional properties of natural polymers can be improved when edible films are combined with natural antimicrobial compounds. Antimicrobial packaging films made from biodegradable and food grade materials, are promising food packaging materials because their proprieties ensure a sustainable development for the modern community.

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