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CURRENT OVERVIEW IN THE FIELD OF APPLICATION OF EDIBLE COATINGS/FILMS (MEAT PRODUCTS EXAMPLES)

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ABSTRACT

Preservation and storage of food is an integral part of the food industry. There is currently a progress in the field of preservation and storage technologies, which reflects the demands made by consumers. Active and intelligent packaging is an innovative technique that offers a wide range of possibilities for improving the quality of the food. In accordance with the green policy and the demands of consumers for the minimum addition of chemical additives to food, there is an open space for the application of edible coatings/films as packaging materials for food. Edible coatings/films due to their natural character are able to act as carriers of wide range of bioactive substances, dyes, nutraceuticals, which can extend the shelf life of food and, at the same time, preserve or improve the quality of food. From these substances, the focus is on phytochemicals, nanoparticles, probiotics, protein hydrolysates or bioactive peptides and others from wide range of sources. The possible incorporation of bioactive peptides with a wide range of bioactive effects is a current topic that attracts the attention of researchers.

Keywords: bioactive peptides, edible coatings, biopolymers, food preservation

INTRODUCTION

Currently, with the constant growth of the living standards of the population, the requirements for the quality, freshness, and health safety of food with high nutritional value, with a minimum content of necessary or as little as possible chemical preservatives and additives are increasing **(Ren et al., 2021)**. Meat is one of the best sources of essential nutrients for the human body, especially with regard to the representation of complete proteins and minerals **(Luong et al., 2020)**. For this reason, but also for specific sensory properties, meat is often the preferred food, worldwide annual meat production is estimated at more than 340 million tons **(Ritchie, Roser, 2017)**. On the other hand, the high nutritional nature of meat makes it a perishable food. The causes of rapid spoilage of meat are microorganisms, enzymatic agents in meat, such as lipases and proteases as well as natural oxidative processes **(Pellissery, et al., 2020)**. Annually, approximately one third of global food production, is turned into bio-waste. That third is approximately 1.3 billion tons. This amount is calculated as a loss of 1.0 trillion US dollars per year **(X. Zhang et al., 2021)**. When it comes to the meat industry, losses due to deterioration and spoilage of raw meat reach 40% of the total production, which represents huge economic losses, it is for these reasons that great emphasis is placed on the effective preservation of raw meat **(Ren et al., 2021)**.
Common methods of preserving raw meat include the use of preservatives, low temperature preservation, high pressure treatment, irradiation, modified atmosphere packaging, and others. The use of these methods, especially with regard to physical methods, requires extensive equipment and financial investment, not to mention the occupation of the storage space. In contrast, preservation using chemical preservatives represent a quick, simple, and easy-to-implement approach to ensure a longer shelf life of products. However, current consumer demands are more towards preferring organic products and natural products, which is the reason for the declining popularity of chemical preservatives **(Zhang et al., 2021)**. For these reasons, we are registering an increase in the introduction of innovative preservation and packaging techniques **(Chen et al., 2012; Rahman et al., 2018)**. A significant part of the research focuses on innovative ways of packaging food, which is a group of packaging technologies using so-called intelligent packaging; active packaging; edible coatings and films; biodegradable packaging or packaging made of nanomaterials **(Chen et al., 2012; Barbosa et al., 2021)**.
This review article discusses the possibilities of using edible coatings/films as biopolymers enabling the incorporation of a wide range of bio-active substances, especially with regard to bio-active peptides with the aim of preserving food products.

CHARACTERISTICS OF EDIBLE COATINGS/FILMS

Edible coatings/films are biopolymers researched and used for food preservation and packaging. This is a technique that takes food packaging to the next level, as the boundaries between food, packaging and preservation disappear here. Here, these three terms, food, packaging, and preservation are combined into one resulting coating/film. The terms edible film and edible coating are often confused. A film is defined as a separate packaging material and a coating is a thin layer less than 0.3 mm thick that is formed directly on the surface of the food. There are several ways of applying films and coatings to food. By soaking the food in a solution, painting it, or creating a separate film, which is then applied to the food. The edible films and coatings in question are produced from individual biopolymers or their combination, these are mainly proteins, polysaccharides, and lipids **(Abdollahzadeh et al., 2021)**. These polymers are obtained from plant sources, animal sources, or products of microorganisms. From the group of carbohydrates, these are mainly cellulose derivatives, starches, carrageenans, pectins, alginates and chitosan. Among the proteins, soy proteins, wheat gluten, corn zein, sunflower proteins, gelatin, casein, collagen, and whey dominate. Since these materials are hydrocolloid, several types of oils and fats are incorporated into the matrix, such as waxes, triglycerides, acetylated monoglycerides, free fatty acids and vegetable oils **(Galus et al., 2020)**. The advantage of edible films and coatings is the possibility of incorporating several substances with antimicrobial and antioxidant effects into their structure, thereby increasing their preservation ability. Edible coatings and films can be made from materials that naturally have a certain spectrum of antimicrobial properties, such as chitosan, which, however, are often enriched with other components such as polyphenols, enzymes, bioactive peptides, essential oils, etc. **(Chawla et al., 2021; Paidari et al., 2021; Song et al., 2021)**.
After selecting the ingredients, the basis of film/coating preparation is mixing with a solvent until complete dissolution. Subsequently, a plasticizer (glycerol, monoglycerides, glucose, polyethylene glycol, etc.) is added to the mixture, as well as a functional component (antioxidants, antimicrobial substances, etc.). The pH value of the solution is adjusted, and the mixture is heated until a homogeneous solution is formed. After subsequent cooling, the aforementioned techniques can be used, i.e., directly immersing the food in the solution, or wrapping the food with foil obtained by drying the solution. When immersion is chosen, the excess solution is allowed to drip from the food and then, the food is dried under controlled conditions, the same procedure is also applied when spraying. In case of foil wrapping, the solution is allowed to dry to form the desired film, which is simply used to wrap the food directly **(Tkaczewska, 2020; Umaraw et al., 2020; Song et al., 2021)**. The resulting physical properties of the coating/film depend on several factors. The original source, the structural organization of the polymer chain, the

chosen processing technology, including the drying conditions as well as the degree of cross-linking or crystallinity **(Galus et al., 2020)**.

BIOPOLYMERS USED FOR THE PREPARATION OF EDIBLE COATINGS/FILMS

As mentioned above, edible coatings/films are produced from individual biopolymers or their combination. These are polysaccharides, proteins, and lipids. The initial origins of the application of edible coatings/films in the food industry date back to the 12th century in China, when various types of waxes were used to preserve citrus fruits and improve their visual appearance by adding shine. Later, fats, especially lard, began to be used in England to extend the shelf life of meat products, followed by lipid coatings in the US in the 16th century to control moisture loss, later replaced by paraffin and harbaub wax. At the beginning of the 20th century, edible coatings were used to add shine and prevent water loss in vegetables and fruits, as casings for sausages and sausages, or as a top layer for confectionery. Nowadays, several biopolymers are used on the same principle, which is to preserve food and improve its quality **(Barbosa et al., 2021, 2022)**.

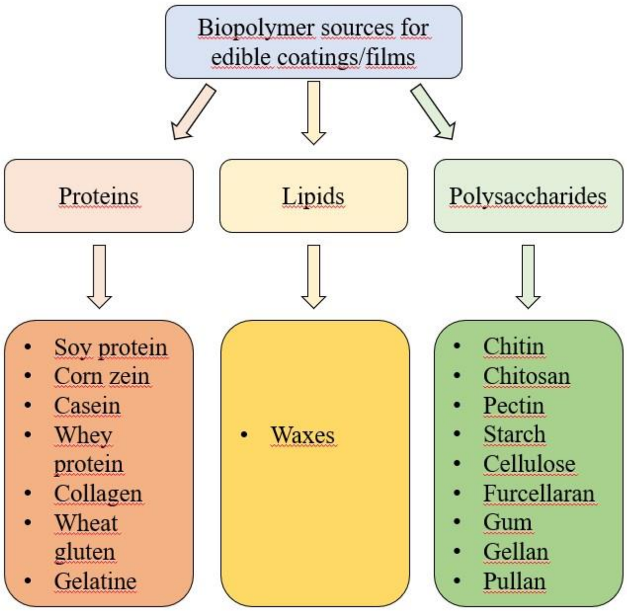

Figure 1 Scheme of biopolymers used for the preparation of edible coatings/films

Polysaccharides

A wide range of polysaccharides such as starch, cellulose derivatives, carrageenans, chitin, chitosan, pectins and others are used to produce edible coatings/films. Polysaccharides are non-toxic materials, characterized by a colorless appearance, serve as a good barrier for carbon dioxide and oxygen, but they do not have the required properties in the case of water vapor transmission. Due to the often-fragile structure of the resulting coatings/films, it is necessary to incorporate plasticizers in the form of, for example, glycerol into the matrix of the coatings/films. Starch is a naturally occurring polysaccharide found in cereals, legumes, potatoes, etc. Starch is composed of several units of 1,4-α-D glucopyranosyl units, more specifically amylopectin and amylose **(Song et al., 2021)**. Starch has been considered a universal biopolymer used in the production of organic packaging due to its gelatinizing properties for decades **(Díaz-Montes & Castro-Muñoz, 2021)**. Starch coatings provide a suitable barrier to oxygen, however, the speed of oxygen transfer through the starch coating depends on the conditions of relative humidity, where at higher relative humidity, there is a faster transfer of oxygen. This is due to the increased mobility of the polymer chain. Coatings and films made from starch are brittle, which can be additionally modified by adding plasticizers such as glycerol, sorbitol, or xylitol **(Tyagi et al., 2021)**. The positive effect achieved by starch coatings is observed especially in the case of fruit preservation, either with or without an incorporated antimicrobial agent **(Garcia et al., 2012; Sánchez-Ortega et al., 2016; Islam et al., 2019)**. Chitosan as a linear polysaccharide contains (1,4)-linked 2-aminodeoxy-β-D-glucan, a derivative of partially deacetylated chitin. It is one of the most abundant naturally occurring polysaccharides and is considered to be non-toxic, biodegradable, functional, biocompatible and characterized by antimicrobial effects **(X. Zhang et al., 2021)**. Chitosan can be obtained by alkaline deacetylation of chitin, which is part of the shells of shrimps, crayfish, and other fishery products. It is insoluble in water, completely soluble in organic acids such as lactic acid and formic acid. The antimicrobial properties of chitosan have been demonstrated against a wide range of bacteria and microscopic fungi. The results demonstrate the antifungal properties of chitosan against microscopic fungi such as *Botrytic cinerea, Fusarium oxysporum, Drechstera sorokiana, Micronectriella nivalis, Piricularia oryzae, Rhizoctonia solani* or *Trichophyton equinum*. Chitosan showed bacteriostatic and bactericidal effects, e.g. against, *E. coli, A. tumefaciens, B. cereus, C. michiganence, K. pneumonia, M. luteus, P. fuorescens* a *X. campestris* **(Paidari et al., 2021)**. The presumed mechanism of chitosan's antimicrobial action is related to electrostatic forces between protonated amino groups in chitosan and negatively charged residues on the cell surface. Chitosan shows a high degree of biocompatibility, acts as a carrier of minerals, vitamins, polyphenols, or other functional substances, which, together with its antimicrobial properties, makes it an excellent packaging material **(Pinzon et al., 2020; Rezaeifar et al., 2020; Xiong et al., 2020a; Zam, 2019)**, the only disadvantage is poor solubility in water **(X. Zhang et al., 2021)**. Chitosan coatings and films are selectively permeable to gases (O2 and CO2) and exhibit desired mechanical properties. These films/coatings exhibit high permeability to water vapor, which limits their use in humid environments, however, as chitosan is biocompatible, it is possible to incorporate other substances into the matrix to eliminate deficiencies or enhance existing properties **(Nair et al., 2020)**. Furcellaran is a natural sulfated anionic polysaccharide obtained mainly from the red algae *Furcellaria lumbricalis*. In its properties, furcellaran is similar to agar or carrageenan. Furcellaran is biocompatible, non-toxic, biodegradable, water-soluble and able to form a gel. It is registered as a natural food additive under the code E407 **(Song et al., 2021)**. As such, furcellaran does not exhibit any antioxidant or antimicrobial properties compared to the aforementioned chitosan. However, like chitosan, it is biocompatible and various antioxidant and antimicrobial agents can be successfully implemented into it in the production of edible coatings/films. The combination of furcellaran with the addition of essential oils, protein hydrolyzates, gelatin and nanocomposites, bioactive peptides for the purpose of producing edible coatings/films, which showed an effect against microbial growth and oxidation processes in food matrices, is observed **(Kulawik et al., 2022)**. Cellulose, as a natural biopolymer, is contained in a wide variety of plants. It consists of d-glucose units that connect β-1,4 glycosidic bonds. Cellulose derivatives are represented by methylcellulose, carboxymethylcellulose, hydroxypropylmethylcellulose and hydroxypropylcellulose, which are obtained by its esterification. Coatings and films made from cellulose derivatives are generally flexible, transparent, tasteless and odorless, water-soluble, resistant to fats and oils, and moderately resistant to oxygen and water diffusion **(Barbosa et al., 2022)**. Cellulose as a component of edible coatings/films is a suitable carrier of bioactive substances, applicable to products of both animal and plant origin **(Kumar et al., 2021; Mousavi et al., 2021; Ruan et al., 2019; Tumbarski et al., 2019)**. Ripe fruits such as apples or currants contain an amorphous, colloidal carbohydrate of white color with a high molecular weight, pectin. Due to its thickening, emulsifying and gel-forming properties, it has been used for a long time in fruit jelly, medicines, and cosmetics **(Valdés et al., 2015a)**. Pectin is largely composed of linear homo-galacturonan chains with branched rhamnogalacturone chains, where the neutral sugar branches are connected through rhamnose residues. Pectin, as well as the other listed polysaccharides characterized by gel-forming properties, are suitable for mixing in various combinations with the possibility of enrichment with bioactive components to achieve the desired functions **(Eça et al., 2015; Muñoz-Almagro et al., 2021; Panahirad et al., 2020; Valdés et al., 2015b)**.

Proteins

Coatings and films made from proteins are superior in terms of mechanical properties compared to polysaccharide coatings/films due to their unique structure. They are characterized by good gas barrier properties and poor water vapor permeability, due to the presence of hydrophilic groups in their structure **(Song et al., 2021; Tkaczewska, 2020)**. Due to extensive intermolecular forces involving the interaction of protein chains, these coatings/films are fragile, one way to reduce their fragility is to incorporate plasticizers into the coating/film matrix. Plasticizers reduce intermolecular forces along protein polymer chains, resulting in increased polymer chain mobility and greater flexibility. The second possibility is to reduce the molecular weight of the polymer by means of enzymatic hydrolysis, thereby achieving less need for the use of plasticizers and at the same time ensuring the required flexibility of the coating/film **(Tkaczewska, 2020)**. From protein groups, milk proteins, collagen, gelatin, soy protein, corn protein and others are used for the preparation of edible coatings/films **(Ananey-Obiri et al., 2018; Chawla et al., 2021; Song et al., 2021; Suhag et al., 2020; Tkaczewska, 2020)**. Milk proteins, such as casein and whey protein, are used not only because of their properties for the preparation of edible coatings/films, including non-toxicity, nutritional value and particularly good biocompatibility **(Galus et al., 2021)**, but also because of the high production of dairy products, in which the required proteins are obtained from by-products such as whey. Whey protein generally consists of five fractions, namely β-lactoglobulin, bovine serum albumin, glymacropeptide, α-lactoalbumin and immunoglobulin. In addition, whey protein also contains some fractions with a low representation of lactoferrin, lactoperoxidase, lysozyme, proteose peptones or osteopontin. As it is an animal protein, its composition can vary slightly depending on the animal and its breeding, which ultimately affects emulsification and other functional properties. Commercially, whey protein is supplied to the market in two forms: a concentrate and a hydrolyzate **(Barbosa et al., 2022; Kandasamy et al., 2021)**. Casein occurs in the form of micelles, 93% of which are casein and the remaining 7% are casein salts with sodium, calcium, magnesium, potassium, and other minerals. Casein is composed of four monomers, namely αS1, αS2, β and κ casein. The advantages of milk proteins in the production of edible coatings/films are their complex intermolecular bonds, which make them excellent gas barriers **(Mishra et al., 2022)**. Among the protein sources, we cannot fail to mention collagen obtained mainly from the connective tissues of animals, the hydrolysis of which also produces gelatin. These proteins have been used as packaging materials in the food, pharmaceutical and cosmetic industries for a considerable time. Perhaps the biggest disadvantage of gelatin is its susceptibility to higher temperatures. Gelatin also has slightly worse barrier and mechanical properties compared to other materials. On the other hand, collagen and collagen coatings are widely known for coating meat products, which retain their properties even during heat treatment **(Hashim et al., 2015) (Lacroix & Vu, 2014; Song et al., 2021)**. Some of the purified grain and pulse proteins are characterized by a unique ability to form an edible coating/film. Among legumes, it is soy protein **(Hoseiniyan et al., 2020; Nandane & Jain, 2015; L. Zhang et al., 2018)**, which is overall one of the most widely used functional components of a polymeric nature **(Yb & Kola, 2019)**. Another protein used to prepare the mentioned coatings/films is zein contained in corn. After drying, it is characterized by its glossiness and the surface is resistant to grease and is insoluble in water, except for waters with a very low or high pH. Zein consists of a group of prolamins that are soluble in alcohol, the advantage of zein is that it is resistant to bacteria and has thermoplastic properties **(Chhikara & Kumar, 2022)**. A potential disadvantage of coatings/films prepared from proteins is their risk for people suffering from selected allergies to individual groups of proteins **(Yb & Kola, 2019)**.

Lipids

Among the lipids, the group of waxes is the most represented in the preparation of edible coatings/films, whether it is waxes from paraffin, beeswax, carnauba and others. From lipid groups, essential oils are also incorporated into edible coatings, but they do not represent the main component of the coating/film, but often a bio-active component added for its antioxidant and other bioactive properties **(Aayush et al., 2022; Chhikara & Kumar, 2022)**. Lipids are particularly suitable compared to proteins and polysaccharides in terms of moisture migration, which is why they are added to coatings/films in combination with proteins and polysaccharides to improve their barrier and mechanical properties. In general, edible coatings/films prepared from lipids are thicker compared to those from proteins and polysaccharides, precisely because of their hydrophobic properties, which can deter potential consumers, so they are preferred for consumption, only in the case of the thinnest possible layer **(Al-Tayyar et al., 2020; Barbosa et al., 2022)**.

EDIBLE COATINGS/FILMS AS ACTIVE FOOD PACKAGING

Active packaging is a system/technology described in the US as a packaging system that protects food from contamination or spoilage by creating a barrier against external conditions while interacting with the internal environment to control the atmosphere within the package **(Fang et al., 2017)**. Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC defines active materials and objects in contact with food as follows:" Active food contact materials and articles (hereinafter referred to as active materials and articles) means materials and articles that are intended to extend the shelf-life or to maintain or improve the condition of packaged food. They are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food". The regulation further states: " Active food contact materials and articles are designed to deliberately incorporate 'active' components intended to be released into the food or to absorb substances from the food. They should be distinguished from materials and articles, which are traditionally used to release their natural ingredients into specific types of food during the process of their manufacture, such as wooden barrels" . Special requirements for active packaging materials are listed in Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. There are many types of active packaging. The basic ones are antimicrobial active packaging and antioxidant active packaging. Antimicrobial active packaging can be created in the four most common ways. The first method is to put antimicrobial substances in a bag or pad inside the package. The second method is the incorporation of antimicrobial substances into the packaging film either by heat treatment by co-extrusion of packaging films with antimicrobial substances, however, thermal incorporation may reduce the antimicrobial effect of the given substances. An alternative is to incorporate antimicrobial substances into the film by mixing solvents, electrospinning, or casting. The third method is coating the package with a matrix acting as a carrier of antimicrobial substances, and the last method is the use of polymers that are inherently antimicrobial, such as use of chitosan. There are two basic groups of antioxidant active packaging, the first is independent antioxidant devices, examples are various bags, pads or labels that contain oxygen scavengers. The second category is separate antioxidant packaging materials **(Fang et al., 2017; Tyagi et al., 2021)**. Since in the case of edible coatings/films, which, as mentioned above, are biocompatible and it is possible to incorporate a wide range of bio-active substances into them **(Paidari et al., 2021)**, as we come to another term, Active edible packaging, which **Barbosa et al. (2021)** defines as: „*food packaging made of comestible bioproducts and active compounds that interacts with the food. The bioproducts, usually biopolymers, must be recognized as safe and with characteristics to be consumed by humans—comestible—and not toxic and capable of carrying an active compound, like anti-browning agents, colorants, flavors, nutrients, antimicrobial and/or antioxidant compounds, in order to extend the product shelf-life, reduce contamination and maintain or even enhance the nutritional value*". Current research points to a wide spectrum of bio-active substances incorporated into edible coatings/films with the aim of subsequent application to the surface of the food and its quality improvement and shelf-life extension **(Alves et al., 2018; Ruan et al., 2019; Tumbarski et al., 2019; Ali et al., 2019; Farhan & Hani, 2020; Pinzon et al., 2020; Rezaeifar et al., 2020; Xiong, Chen, et al., 2020; Kulawik et al., 2022).**

BIOACTIVE SUBSTANCES AS PART OF EDIBLE COATINGS/FILMS

Antimicrobial and antioxidant effects are the most common desired effects, due to which bioactive substances are incorporated into edible coatings/films **(Salgado et al., 2015)**. The antimicrobial effect may not always be achieved only with the external bioactive substance in the coating/film matrix, but in some cases the biopolymer itself, from which the coating/film is prepared, is characterized by these effects, as it is for example in the case of chitosan **(X. Zhang et al., 2021)**. Of the substances characterized by bioactive properties, essential oils, polyphenols, bacteriocins, bioactive peptides, enzymes, anthocyanins, nanoparticles, plant extracts, probiotics, colorants, and others are added to edible coatings/films, depending on the requirements placed on the individual coating/film **(Quirós-Sauceda et al., 2014; Salgado et al., 2015; Umaraw et al., 2020)**. Bioactive compounds are non-nutritive substances that occur in small quantities, they can be found in a wide range of plant species, are produced by representatives of microorganisms, and are also contained in selected species of animals and marine organisms. As a rule, they are obtained by extraction methods and are subsequently incorporated into the matrix component of the prepared coating/film solution **(Quirós-Sauceda et al., 2014)**.

Plant bioactive compounds incorporated into edible coatings/films

Secondary metabolites of a wide range of plants, starting from exotic species, medicinal plants, herbs, spices, fruits, vegetables are a rich source of a large number of phytochemicals with bioactive effects **(Zhao et al., 2015)**. To this date, more than 5,000 bioactive plant compounds, so-called phytochemicals, have been recorded, which are divided into polyphenols, alkaloids, terpenoids, phytosterols and organic sulfur compounds, of which polyphenols are the most studied **(Bayir et al., 2019)**. Polyphenols consist of one or more hydroxyl groups (OH) attached directly to an aromatic ring. They are characterized by antioxidant, antimicrobial, immunomodulating and anti-inflammatory effects, which is the reason for long-term research in the field of medicine, food, and pharmaceuticals **(Altemimi et al., 2017; Zhao et al., 2015; Bayir et al., 2019)**. Polyphenols are further divided into four main groups, flavonoids, phenolic acids, stilbenes and lignans **(Lee et al., 2017)**. Antimicrobial and antioxidant effect is required in the preparation of edible coatings/films applied to meat and meat products **(Umaraw et al., 2020; Song et al., 2021)**, the antimicrobial effect of bioactive components of plants is ensured by the presence of phenolic compounds and their structural diversity, the antimicrobial effect has been proven by several studies against a wide range of bacteria, microscopic fungi, but also viruses **(Christaki et al., 2012; Rawdkuen, 2019; da Silva et al., 2022)**. The antioxidant effect of bioactive plant components is ensured by the fact that they act as chelating agents, inhibit binding to metal ions, decompose single oxygen, hydroperoxides and reactive components. They also additionally ensure the reduction of free radicals by donating electrons **(Vuolo et al., 2018)**. Despite these desired effects, plant bioactive compounds have limited use in the preparation of edible coatings/films due to their strong sensory attributes that can change the expected sensory properties of meat and meat products, as is the case for example of essential oils, which are characterized by their specific strong aroma **(Barzegar et al., 2020)**. More detailed elaboration of the mechanisms of the effects of phytochemicals and their use is presented in a previous work by **Vlčko et al. (2022)**. Specific examples of edible coatings/films enriched with plant bioactive compounds applied to several types of meat and meat products with observed effects are listed in Table 1.

Table 1 Examples of application of edible coatings/films enriched with plant bioactive compounds to meat and meat products

Biopolymer	Bioactive compound	Meat/meat product	Observed effect	References
Chitosan	Tomato plant extract	Pork loin	Antimicrobial effect at all concentrations of the extract. The best antioxidant effect at a concentration of chitosan 1% and extract 0.3%. The highest acceptability of the product at a chitosan concentration of 1% and an extract of 0.1%. Extending the shelf life of meat samples by 13 days at a concentration of chitosan 1% and extract 0.1%.	**(Chaparro-Hernández et al., 2019)**
Lepidium sativum seed mucilage	Essential oil of *Heracleum lasiopetalum*	Fresh beef	Statistically significant increase in oxidative and microbiological stability and acceptability of samples during storage for 9 days at 4 °C.	**(Barzegar et al., 2020)**
Chitosan, whey protein	Quince and cranberry juice	Fresh turkey pieces	No observed synergic effect. Microbiological protection against the development of pathogenic microorganisms E. coli, C jejuni, S. typhimurium for 6 days at different temperatures.	**(Brink et al., 2019)**
Chitosan	Clove oil	Cooked pork sausages	Inhibition of microbial growth. Slowing down of lipid oxidation. Extending the shelf life of samples coated with a coating with a concentration of 2% chitosan and 1.5% clove oil by 6 days compared to control samples at a storage temperature of 4 ± 2 °C.	**(Lekjing, 2016)**
Whey protein	*Origanum virens* essential oils	Traditional Portuguese sausages (paínhos alheiras)	Protection against color degradation observed in paínhos samples. Significant reduction of lipid peroxidation in alheiras samples. Inhibition of microbial load in both cases. Extending the shelf life by 20 days (paínhos) and 15 days (alheiras) with an essential oil concentration of 1% at a sample storage temperature of 4 °C.	**(Catarino et al., 2017)**
Alginate	Capsular form of *Terminalia arjuna*	Chevon sausages	Significant improvement in oxidative stability at *Terminalia arjuna* concentrations of 0.5 and 1% at a storage temperature of 4 °C. Significant improvement of microbial quality at *Terminalia arjuna* concentration of 0.5 and 1% at storage temperature of 4 °C. Significant increase in sensory score at concentrations of 0.5 and 1% of *Terminalia arjuna*.	**(Kalem et al., 2018)**
Pectin	Resveratrol nanoemulsion and oregano essential oil	Pork loin (MAP)	Significant extension of shelf life Partial inhibition of lipid oxidation. Partial inhibition of protein oxidation. Inhibition of microbial growth.	**(Xiong, Li, et al., 2020)**
Glucomannan, carrageenan	Camellia oil	Chicken meat	Partially inhibited oxidation of lipids and proteins. Inhibition of microbial growth. Extension of shelf life to 10 days at a concentration of Camellia oil 3.5% at a storage temperature of 4 °C. Not observed aroma affect of the samples.	**(Zhou et al., 2021)**
Pectin	Essential oils and extracts of *Thymbra spicata* and *Thymus vulgaris*	Sliced bolognas	Delaying the growth of total aerobic bacteria, lactic acid bacteria, microscopic fungi in the case of essential oils. No observed antioxidant activity against lipid oxidation in the case of extracts. Extension of shelf life by 6 days in case of application of essential oils. Antimicrobial effect without negative impact on the sensory quality of the samples in the case of essential oils with concentrations of 0.5 and 0.5%.	**(Gedikoğlu, 2022)**
Alginate	Complex of hydroxyapatite-quercetin and quercetin glycosides	Chicken fillets	Slowing of degradation processes during storage of samples at °6 C for 11 days. Inhibition of microbial growth. Inhibition of increase in TVB-N values.	**(Malvano et al., 2022)**
Gelatine	*Lawsonia inermis* extract	Beef meat	Increasing of shelf-life of beef meat stored at 4 °C. Partially inhibited oxidation of lipids. Color preservation. Inhibition of microbial growth (total bacteria count, psychrophilic bacteria count). Slowing down the proteolysis process.	**(Jridi et al., 2018)**
Soy protein isolate	Thyme and oregano essential oils	Fresh beef	Best antimicrobial effect against *E. coli* O157:H7, *L monocytogenes*, *S. aureus* at 3% thyme/oregano oil concentration. Decrease in sensory acceptance with increasing concentrations of essential oils. Total acceptance at all essential oil concentrations (1%, 2%, 3%).	**(Yemiş & Candoğan, 2017)**
Curdlan/Chitosan	Tea polyphenols	Fresh black pork tenderloin	Greater inhibition of microorganisms after the addition of tea polyphenols. Observed antioxidant effect. Reduction of water vapor permeability coefficient.	**(Zhou et al., 2019)**
Chitosan	Gallic acid	Fresh pork in MAP package	Increased preservative effect on fresh pork stored in a modified atmosphere at 4 °C. Decreased lipid and myoglobin oxidation. Antimicrobial effect. Better preservation of the meat texture. Pro oxidative effect of proteins at 0.4% concentration of gallic acid. Optimum gallic acid concentration is needed. 2% concentration of gallic acid without pro-oxidant effect	**(Fang et al., 2018)**

Other bioactive compounds incorporated into edible coatings/films

Bioactive substances from other sources, such as plants, are also added to edible coatings/films in order to improve their effectiveness. These are chemical preservatives, enzymes produced by animals and microorganisms **(Wang et al., 2017; Wu et al., 2018; Rawdkuen, 2019; W. Zhang & Rhim, 2022)**, bioactive substances obtained from the marine environment **(Deepika et al., 2022)** or even probiotics **(Pop et al., 2020)**. A special part is nanotechnologies and nanomaterials, which can be created from animal, plant or inorganic sources and incorporated into the coating/film structure **(Zambrano-Zaragoza et al., 2018; Umaraw et al., 2020)**. The most applied enzyme in the matrix of coatings/films with antimicrobial effects is lysozyme. Lysozyme is characterized as a nutraceutical, it is made from egg white, blood, and milk. Its antimicrobial effects are proven against a wide range of microorganisms, especially against gram-positive bacteria by hydrolysis of 1,4-beta-bonds between N-acetylmuramic acid and N-acetylglucosamine in the cell wall **(Rawdkuen, 2019; Khorshidian et al., 2022)**, its use in the preparation of edible coatings/films, when separate better mechanical properties and better barrier properties against water vapor have been demonstrated in studies **Hu et al. (2022)**; **Koca & Bayramoğlu (2022). Kaewprachu et al. (2015)** applied an edible gelatin-based film with an incorporated combination of catechin and lysozyme to ground pork stored for 7 days at 4 °C. Meat wrapped in the subject film showed less weight loss, less color degradation, as well as lower TBARS values compared to meat wrapped in PVC film. Also, meat wrapped in an edible film enriched with a lysozyme-catechin combination more effectively inhibited the microbial growth of the total number of bacteria as well as microscopic fungi. Lactoperoxidase is another of the group of enzymes with desirable antimicrobial effects **(W. Zhang & Rhim, 2022)**. **Yousefi et al. (2018)** investigated the effect of alginate coatings containing a lactoperoxidase system at concentrations of 2, 4 and 6% on the shelf life of chicken meat stored at 4 °C for 16 days. The coating in question with an incorporated lactoperoxidase system was compared in terms of its effect on extending shelf life with a coating without the included lactoperoxidase system. The results of the study show a more favorable effect especially of coatings containing 6% of the lactoperoxidase system on reducing the formation of TVB-N and inhibiting the growth of microorganisms compared to control samples, at the same time, the coating containing the lactoperoxidase system with a concentration of 6% showed acceptable sensory properties. In their study point to the beneficial antimicrobial effect of a protein-alginate edible coating with an incorporated lactoperoxidase system at concentrations of 2, 4 and 6% on total aerobic mesophilic bacteria, *Pseudomonas aeruginosa* and *Enterobacteriaceae* analyzed on chicken meat stored for 8 days at 4 °C, while with increasing concentration of the lactoperoxidase system, the expected better antimicrobial effects also occurred. There are countless organisms in the marine environment that differ in their physiology and adaptation, the adaptive processes of marine organisms ensure the production of bio-active substances but also new species of organisms producing new substances. For this reason, the beginning of extensive research in the field of bioactive substances of marine origin dates to 1960. The result of research activities in this area is also the fact that between 2005 and 2010 more than 10,000 marine metabolites were isolated and characterized **(Bhatnagar & Kim, 2010)**. Within the framework of a sustainable economy, fishing waste can serve as a source of bio-active substances and materials with bio-active effects suitable for the preparation of edible coatings/films (Naga Deepika et al., 2022). An excellent example is the use of chitosan mentioned above, which is pointed out in the study **Jamróz et al. (2021)**, or collagen hydrolysates from fish in **Gomez-Guillen et al. (2011); Tkaczewska (2020)**. Nanoparticles incorporated into the structures of coatings/films of organic and inorganic origin are a special part **(Zambrano-Zaragoza et al., 2018)**. Nanoparticles are used for their antimicrobial effects in several branches of industry, these are mainly nanoparticles of gold, zinc, silver, copper or selenium alone or in the form of oxides, or others. Their antimicrobial activity is attributed to their size, shape and surface properties. Nanoparticles also have an antimicrobial effect thanks to the mechanism of production or catalysis of reactive oxygen species, which diffuses into the surface of the material fiber and reacts with a foreign organism there, leading to DNA degradation, lipid peroxidation, protein oxidation, and ultimately cell death. On the other hand, the use of nanomaterials is accompanied by several controversies from the point of view of potential negative effects, which are still the subject of research **(Staroń & Długosz, 2021)**. From organic materials, chitosan can be mentioned again, which also in nano form finds application in the preparation of edible coatings/films, but also other biopolymers in the form of nanofibers presented in studies **Zambrano-Zaragoza et al. (2018); Rosyada et al. (2019); Kumar et al. (2020); Staroń & Długosz (2021). Meindrawan et al. (2020)** applied an edible coating based on a polymer bionanocomposite of gelatin and zinc oxide nanoparticles to broiler chicken fillets left at room temperature for eight hours. The edible coating containing 0.048% of zinc oxide nanoparticles per 100g solution had a significantly higher antimicrobial effect compared to the control sample without incorporated nanoparticles, the edible coating also preserved the sensory properties of the meat for a period of time without signs of slime formation, rancidity or other undesirable changes while ensuring a stable pH of 6 reduced the water loss of the sample and showed stable barrier properties against water vapor transmission. Probiotics are characterized by their health-promoting properties in relation to the gastrointestinal tract and intestinal microflora. Probiotic cultures can be incorporated into edible coatings/films in order to enrich this coating/film with beneficial effects on health, but also for their inhibitory effects against a wide range of pathogenic bacteria and food spoilage bacteria, as reported in studies **Pavli et al., (2018); Pop et al. (2020)**.

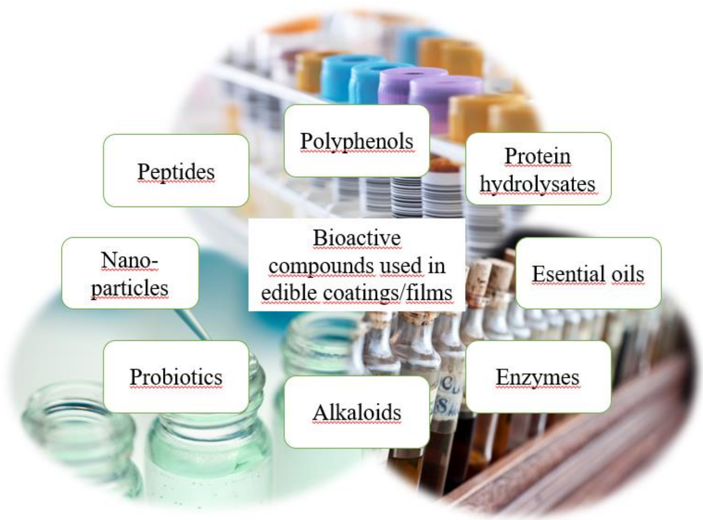

Figure 2 The most used bioactive compounds in edible coatings/films

USE OF BIOACTIVE PEPTIDES

Biologically active peptides can be natural food compounds or parts of a protein that are inactive in the precursor molecule, but are active when released or transported to the active site. In humans, peptides are generally the result of enzymatic hydrolysis of consumed protein in the gastrointestinal tract. Biopeptides are also products of microorganisms mainly through fermentation activity. A wide range of proteases are used for proteolysis to obtain biopeptides with specific activity. When extracting biopeptides from plant tissues, e.g. ficin, papain, bromelain; in the case of animal tissues, this is pepsin, chymotrypsin, or trypsin, and in the case of microbial cells, enzymes such as proteinase K, prionase, collagenase, subtilisin A, Alcalase®, Flavorzyne®, or Neutrase® can be used **(Jakubczyk et al., 2020)**. Obtaining peptides by enzymatic hydrolysis is more advantageous compared to microbial fermentation, as the hydrolysis process is faster, more predictable, and easier to scale. Mostly peptides with a shorter chain are obtained by enzymatic hydrolysis. Short chain peptides show better antioxidant properties compared to longer chain peptides **(Daliri et al., 2017)**. Obtaining peptides using the fermentation activity of microorganisms involves the cultivation of selected species of yeast and bacteria on protein substrates, in order to hydrolyze proteins using microbial enzymes. Growing yeast and bacteria secrete their proteolytic enzymes into the protein material, ensuring the release of the peptide from the parent protein. As a rule, the selected bacterium is left in the broth until reaching its exponential phase at the desired temperature, then the cells are collected, washed, and suspended in sterile distilled water containing glucose, after which they are inoculated into a sterilized protein substrate. The extent of this method of hydrolysis depends on the strain of the microorganism used, the type of protein and the fermentation time **(Daliri et al., 2017)**. Peptides can be produced also by chemical synthesis. The process of chemical peptide synthesis is most often carried out by joining the carboxyl group of the input amylacetic acid to the N-terminus of the growing peptide chain. One of the most preferred current methods of chemical peptide synthesis is solid-phase peptide synthesis (SPPS). This synthetic method takes place under heterogeneous conditions on a polymeric solid support, the process also involves deprotection and washing to remove unreacted groups and by-products. SPPS makes it possible to synthesize peptides using automation, allowing for easier post-translational modifications of the backbone and the insertion of amino acids that are not commonly found in natural peptides. The method can be described as revolutionary, which has provided a wide range of possibilities to create peptides with specific properties, for example, within the pharmaceutical industry in the form of therapeutic agents and vaccines **(Petrou & Sarigiannis, 2018)**. Bioactive peptides have several desired effects, which are used in medicine, cosmetics, or the food industry. Bioactive peptides exhibit antimicrobial, antioxidant, anticoagulation, antiproliferative or immunomodulating properties. Several of them can act as angiotensin-converting enzyme inhibitors (ACE inhibitors) and play a role in hemolytic, opioid, and calcium binding. Evaluation of proteins from the point of view of bioactive peptide precursors is performed based on their potential activity profile and the frequency, with which bioactive fragments occur in the sequence **(Perez Espitia et al., 2012; Tkaczewska, 2020; Peighambardoust et al., 2021)**. In connection with edible coatings/coatings, attention is drawn to bioactive peptides mainly for their antioxidant and antimicrobial effects. The antimicrobial effects of bioactive peptides include antibacterial, antifungal as well as antiviral effects **(Perez Espitia et al., 2012; Santos et al., 2018; Jakubczyk et al., 2020; S. Zhang et al., 2021)**.

Antioxidant peptides

Antioxidant peptides are bioactive peptides with antioxidant activity, most of them consist of two to sixteen amino acids. Antioxidant properties of peptides depend on their amino acid composition, size, method and conditions of their acquisition and degree of hydrophobicity. Studies confirm the trend that the lower the molecular weight of the peptide, the higher the antioxidant activity. Several amino acids are characterized by antioxidant properties, examples are histidine, glutamic acid, proline, tyrosine, cysteine, methionine, or phenylalanine. Amino acids bind prooxidative metal ions to perform their activity, scavenging OH radicals and/or inhibiting lipid peroxidation. Consequently, each individual amino acid contributes uniquely as an antioxidant depending on its type. Peptides that have tyrosine in their chain work primarily through the transfer of hydrogen atoms, while peptides that have cysteine, tryptophan, and histidine in their chain work through the transfer of one electron. Aromatic amino acids such as tyrosine and phenylalanine play an important role in donating protons to electron-deficient radicals, this property can increase the ability of bioactive peptides to scavenge radicals. The antioxidant capacity of peptides containing histidine is directly related to hydrogen donation and trapping of peroxyl radicals in lipids. The sulfhydryl group in cysteines is responsible for the antioxidant properties due to its primary reaction with radicals **(S. Zhang et al., 2021; Zaky et al., 2022)**. The structural properties of the peptide do not only determine its reactivity, but also influence its interactions with the food matrix. In the potential application of a peptide, the priority is to obtain a peptide with the most desired effects, therefore, before the application of the peptide, studies of their bioavailability or expected interactions with a specific food are carried out using in vitro gastrointestinal systems and in silico analyses. Several online free bioinformation tools are available, for example the BIOPEP database, which predicts the biological activity of peptides. It is also appropriate to apply molecular docking simulation models, determine quantitative relationships between structure and activity and structure and properties to characterize the structural properties and physicochemical properties of peptides. Combination of in vitro and in silico analyzes of peptides before potential use, which can take place simultaneously and thereby ensure the best possible estimate of the antioxidant activity of a peptide obtained from food proteins (Jakubczyk et al., 2020).

Antimicrobial peptides

Bioactive peptides show antimicrobial effects against a wide range of bacteria, yeasts, and viruses. Especially with antimicrobial peptides (AMPs), further biological activity is often noted, such as antioxidant effect, immunomodulation, or wound healing activity. The antimicrobial activity of peptides is related to their physicochemical properties, such as size, charge, hydrophobicity, amphipathy and solubility, as well as to the type and number of individual amino acids in the peptide chain. AMPs have around twelve to fifteen amino acids in their chain, AMPs differ from each other not only in the length and representation of amino acids, but also in the charge and position of the disulfide bonds. AMPs mainly have a rich representation of cationic and hydrophobic amino acids and have cationic (positively charged) and amphiphilic (both hydrophilic and hydrophobic) properties. The presence of both hydrophilic and hydrophobic amino acids at the terminals or the presence of a positive charge are recognized as the main structural motifs, through which the interaction between AMP and the microbe occurs. Several peptides with antimicrobial effects have been identified over the years and are available in online databases such as APD3, DRAMP or YADAMP **(Daliri et al., 2017)**. Antifungal peptides (AFP) target the intracellular components of microscopic fungi or their cell wall, which leads to disruption of the integrity of the cell membrane and subsequent change in permeability by the formation of pores in the structure of the given membrane. Several AFPs selectively respond to conserved molecular structures that are unique to the target species. The echinocandin family is a typical example of AFPs that inhibit 1,3-β-glucan synthase. 1,3-β-glucan synthase is essential for maintaining the integrity of the fungal cell wall. In case of inhibition of this function, destabilization of the cell wall occurs, the septal micropore is disturbed and the cells become susceptible to osmotic pressure. β-glucan synthase occurs to a large extent in the case of *Candida, Aspergillus, Cryptococcus* or *Pneumocystis* species **(Moravej et al., 2018)**. The most studied peptides are antimicrobial peptides showing antimicrobial activity against bacteria, the so-called antibacterial peptides (ABP). These are characterized by interaction with the cytoplasmic membrane of the microorganism regardless of the final goal. The physiological mechanism of antibacterial peptides is based on binding to bacterial cell membranes or mitochondrial membranes, resulting in disintegration and subsequent cell death. The mechanism in question is based on the electrostatic interaction between positively charged peptides and negatively charged cell membrane surfaces. ABP subsequently disrupt the continuity and structure of the cell membrane **(Perez Espitia et al., 2012; Jakubczyk et al., 2020)**. Antiviral peptides (AVPs) significantly inhibit viruses at various stages of their life cycle, including entry, attachment, replication, transcription, translation, replication, and release in the host cell. The action of AVP can be divided into 3 main mechanisms, namely, inhibition of the attachment of the virus and its fusion with the cell membrane; disruption of the envelope of target viruses; integration with viral polymerase resulting in inhibition of viral replication **(Perez Espitia et al., 2012; Moravej et al., 2018)**.

Application of bioactive peptides in edible coatings/films

For the above reasons, bioactive peptides are being researched and added to foods. As stated, edible coatings/films can be enriched with bioactive peptides precisely for their antioxidant and antimicrobial or immunomodulating properties, which achieve a better preservation effect and increase the overall value and quality of the given food. Since microbial contamination of foods, including meat, occurs mainly on their surface, the application of AMP-enriched films/coatings may be more effective than their direct addition to the product. Peptides are continuously released from the coatings/films onto the surface of the food, thereby maintaining the concentration. Also, application of AMP to coatings/films requires a smaller amount compared to direct addition of AMP to food. Bioactive peptides can also be combined with other bioactive compounds according to the requirements and expectations of a specific active/smart package. Active packaging with the addition of bioactive peptides can be obtained in three ways. By directly incorporating the peptide into the polymer matrix; by applying the peptide to the polymer surface or by immobilizing the peptide in the polymer **(Perez Espitia et al., 2012; Tkaczewska, 2020)**. In the study by **Venkatachalam & Lekjing (2020)** was evaluated the application of edible chitosan films with an incorporated combination of the bio-active peptide nisin and clove essential oil on pork patties stored at a temperature of 4 °C for 15 days. Edible film with a concentration of 2% chitosan, 6,400 mg/ml essential oil and 204,800 IU/ml nisin. Based on the results of the study, the qualitative changes in the pork patties treated with the film containing nisin and clove essential oil were minimal, overall, the shelf life of the pork patties was extended by 6 days based on microbiological and sensory analyzes compared to the control samples. The essential oil showed stronger antioxidant effects compared to chitosan and nisin, but the combination of chitosan, nisin and essential oil showed a potential synergistic effect. Another study conducted by **Xiong et al. (2020)** investigated the preservative effect of an edible coating consisting of chitosan and gelatin, into which grape seed extract and nisin were incorporated. The edible coating was applied to fresh pork that was stored for 20 days at a temperature of 4 °C. The results show the antimicrobial effect of the coating containing 1% chitosan, as well as the inhibition of oxidation processes, the addition of 3% gelatin improved this effect and the inclusion of 0.5% extract achieved the best preservation effect, the addition of 0.1% nisin no longer influenced increasing the antioxidant and antimicrobial effect of the coating. However, the coating containing chitosan, gelatin and nisin showed better preservation effects compared to coatings with only chitosan-gelatin combination. In study by **Kulawik et al. (2022)** edible coatings consisting of a combination of chitosan (1.8%) and furcellaran (0.2%) were applied separately with the bioactive peptides RW-4 (0.125%) and LL37 (0.25%) for pork ham and meat that were stored for 10 days at refrigerator temperatures. During 10 days of storage, the control samples exceeded the **NSW Food Authority (2009)** microbial load limit. All used coatings inhibited the growth of bacteria and achieved inhibition in the range of 1.60 to 3.70 log cfu/g. The coating containing peptide RW4 showed the greatest antimicrobial activity. The results show the antimicrobial properties of the newly developed coatings. Coatings with RW4 at a dose of 2.5 μg/ml and with LL37 at a dose of 10 μg/ml completely inhibited the growth of microorganisms in pork meat stored under the same conditions as the pork ham samples. Peptide LL37 is of human cell origin and the peptide designated as RW-4 was artificially synthesized. The antimicrobial effects of the human cathelicidin peptide LL37 have been demonstrated e.g., in studies **Duplantier et al. (2013)** and **Sancho-Vaello et al. (2020)**.

EU LEGISLATIVE FRAMEWORK FOR EDIBLE COATINGS/FILMS

Within the European Union, the general requirements regarding materials and objects intended to come into contact with food are listed in Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC, which, however, in point (9), states: "*Covering or coating materials forming part of the food and possibly being consumed with it should not fall within the scope of this Regulation. On the other hand, this Regulation should apply to covering or coating materials which cover cheese rinds, prepared meat products or fruit but which do not form part of food and are not intended to be consumed together with such food"*. We also do not record any mention of edible packaging materials either in Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. The given Regulation establishes requirements for active and intelligent packaging, which, in our opinion, edible coatings/films characterized by demonstrable antimicrobial, antioxidant, or other properties are clearly. In the relevant regulation, active materials and objects are defined as follows: "active materials and articles means materials and articles that are intended to extend the shelf-life or to maintain or improve the condition of packaged food; they are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food" which undoubtedly correlates with the overall principle and purpose of applying edible coatings/films enriched with bioactive ingredients. Since in the end, edible coatings and films are part of the final food product, such component is also under the general legislative requirements related to food based on the Regulation (EC) No 178/2002 of the European Parliament and of the Council and other generally binding EU regulations following this regulation, as well as legislation regarding priority food additives (Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives), as well as legislation regarding novel foods, if such a product has not already been placed on the market (Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods). In the absence of specific EU measures, EU Member States may maintain or adopt their own national provisions on the materials in question in accordance with Article 6 of Regulation (EC) No 1935/2004. The subject matter of defining and assessing edible coatings/films in accordance with the EU legislative framework is quite complex, as we have mentioned above in the work, it is really an innovative technique in which food, preservation and packaging are combined into one whole. Regarding the addition of bio-active peptides to food, they must first of all be approved and listed in the list of additives according to (Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives). To this date, in presented Regulation is mentioned bio-active peptide nisin under the code E 234 **(Younes et al., 2017)**.

CONCLUSIONS

Edible coatings/films made from biopolymers, which are mainly proteins, polysaccharides and lipids of animal and plant origin, break down the boundaries between food, packaging and preservation technology. Due to their biocomposite properties, they act as carriers of a wide spectrum of bioactive substances with a wide range of effects, whether it is antimicrobial, antioxidant, anti-inflammatory, immunomodulating or others. Incorporating natural-based bioactive substances into the structure of edible coatings/films is a suitable and current technique for extending the shelf life and storability of food in accordance with the green policy and circular economy, as well as meeting the requirements and the trend of rejection of chemical preservatives in food by consumers. The current topic is research in the field of application of bioactive peptides, most of the effects of which are still at the level of research, but the information so far points to their promising potential in the field of food preservation.

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