Antimicrobial Food Pads Containing Bacterial Cellulose and Polysaccharides

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Abstract
Antimicrobial food packaging is one of the major innovations in the field of packaging technology. To extend food shelf life and to contribute to the consumer’s health are the main challenges of the new technology. Absorbent pads are

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widely used in food industry in order to preserve sensorial characteristics of packaged fresh or refrigerated food products, such as meat or poultry and also fruit and vegetables which could generate exudates during storage time. Cellulose and cellulose-derived materials are already used as components in food pads architecture. To tailor an antimicrobial food pad using natural antimicrobial agents is also a challenge which could be achieved. The aim of this chapter is to give an overview of antimicrobial packaging, underlying especially the role of natural antimicrobial agents and biopolymers. Examples are focused on cellulose and its derivative uses. In the second part of this chapter, we propose new composite hydrogels composed of bacterial cellulose and other polysaccharides as xanthan and carboxymethylcellulose, hydrogels which could act as super-absorbent of moisture and fluids exuded from packaged fresh food products. As antimicrobial substances we have tested potassium sorbate and thyme essential oil. The samples impregnated with thyme essential oil were tested against four microbial strains: *Escherichia coli*, *Bacillus subtilis*, *Candida utilis* (Torula), and *Penicillium hirsutum*.

### Keywords
Antimicrobial · Food pad · Bacterial cellulose · Carboxymethylcellulose · Xanthan · Moisture absorber

## 1 Introduction

Active packaging is defined as a packaging containing particular components in or on either the packaging material or in the package headspace with the aim to extend shelf life of food and, at the same time, maintain the food quality. An important class of active packaging is antimicrobial packaging. Its role is to inhibit or to retard the growth of the microorganisms by releasing antimicrobial (AM) agents into the food surface. Many substances were tested as antimicrobial agents. The antimicrobial compounds could be either chemically synthesized or natural ones. They could act by diffusion if they are nonvolatile or as released vapors in the headspace of the packaging, if they are volatile substances. Due to the consumers’ concern regarding pollution caused by synthetic substances and especially by synthetic polymers used as packaging materials, the recent trend in the field of antimicrobial packaging is to use natural antimicrobial compounds and biodegradable polymers. Food industry already uses antimicrobial substances to extend shelf life of many products including meat, poultry, cheese, bread, fruit, and vegetables. The incorporation of antimicrobial agents in food is not agreed by consumers, and for this reason the preservative agents must be applied to packaging; therefore developing antimicrobial packaging becomes a necessity. This kind of packaging is more easily accepted by consumers because of their refusal of products containing additives for preservation [1].

Antimicrobial packaging could be used for meat products to minimize the risk of poisoning and spoilage. This industry already uses different preservative systems meant to avoid microbial contamination of meat surface.
For many food products, the main problem is not only the microbial contamination but also the moisture and the fluids exuded by meat, poultry, and fish which are a major cause of food spoilage. These unsanitary juices could be removed by using absorbent pads, sheets, and blankets. For chilled fish large sheets and blankets are recommended, because melted ice must be absorbed [2]. In order to avoid the food contamination by the absorbed juices, antimicrobial moisture regulators have also become a necessity.

This chapter is structured into two parts; the first one is a brief overview of the principles of antimicrobial packaging, especially about antimicrobial food pads. The second one presents experimental work of obtaining and characterization of a new antimicrobial food pad fully biodegradable containing xanthan, carboxymethylcellulose, and bacterial cellulose.

2 Antimicrobial Agents

2.1 Chemically Synthesized Antimicrobial Agents

The first AM agents used for antimicrobial packaging were synthetic, namely, sorbic and benzoic acid and their salts. A very well-known paper belongs to Han and Flores, who examined the mechanism controlling the release of potassium sorbate through various plastic films, in order to obtain an active packaging material [3]. Not only the aforementioned acids and their salts but also other organic acids and their salts are still used as antimicrobial agents (acetic, benzoic, propionic lactic, citric, and malic acids) [4–8]. As matrices in which these organic substances were incorporated, we could mention synthetic polymers like low-density polyethylene, EVA (ethylene-vinyl acetate), and other polyolefins, as well as biopolymers like chitosan, cellulose and cellulose derivatives, soy protein, corn zein, wheat gluten, and gelatine [9–15].

2.2 Nanoparticles Used as Antimicrobial Agents

As a consequence of the growing interest in the field of nanotechnology, a great number of nanoparticles have been used as antimicrobial agents. As nanoparticles there were tested silver nanoparticles (AgNP), titanium dioxide (TiO₂), zinc oxide (ZnO), cuprum oxide (CuO), and magnesium oxide (MgO) [16]. The use of AgNP-loaded packaging materials against microbial growth in foods is very well documented. The AgNPs could be synthesized <ex situ> and <in situ>. In the first approach, the AgNPs are first synthesized and then dispersed into a polymer or biopolymer matrix. In the second approach, the AgNPs are generated starting from precursors in the polymer solution or dispersion. AgNP-loaded materials have been proven effective against different kinds of microorganisms, especially bacteria, even if the acting mechanism of silver particles is not completely understood. Even if research in this field is developing, recently a general concern has been expressed.
among the scientific community with regard to the risk of exposure to nanoparticles which are present in food packaging and could interact with the human organism [16].

2.3 Natural Antimicrobial Agents

The recent trend in the field of antimicrobial packaging is the use of antimicrobial compounds of different origins: vegetal, animal, or obtained from microorganisms. In the first category, one could include essential oils and other plant extracts which have not only antimicrobial properties but also antioxidants ones. Some nanoclays or layered silicates are also antimicrobial agents but of mineral origin.

2.3.1 Essential Oils and Plant Extracts

Plants are a great source of biomolecules which work in synergism, conferring advantages for plants’ survival. Essential oils and other plant extracts are already known for their great antimicrobial and antioxidant potential. They represent secondary metabolites synthesized by plants for their defense against different stress factors. There are several methods for extracting these valuable compounds, namely, steam distillation (especially for essential oils), solid-liquid extraction, and new techniques for extraction enhancement like ultrasound-assisted extraction, accelerated solvent extraction, microwave-assisted extraction, and supercritical fluid extraction. The strong antimicrobial effects of plant extracts are due to their composition, which contains phenolic components (ca. 85%). Phenolic components constitute a major class of secondary metabolites produced by plants. More than 8000 phenolic structures were already identified [17]. These compounds are considered to be essential to the physiology and the cellular metabolism of plants and promote physiological survival of the plant, providing protection against pathogens and predators [18]. From the large class of phenolic compounds, there could be mentioned other subclasses of compounds with antimicrobial and antioxidant properties like flavonoids, phenolic acids, and lignans [19]. The most important members of flavonoids are the flavonols, flavanols, flavones, isoflavones, anthocyanidins or anthocyanins, and flavanones [18]. The antimicrobial and antioxidant effects of phenolic compounds against different pathogenic microorganisms are very well documented [18–20].

Essential oils (EOs) are aromatic oily liquids obtained from different parts of plants. They can be obtained using different methods, steam distillation being the most common in what commercial products are concerned. A great number of EOs is known, but only 300 of them are commercially relevant, especially those destined for flavor and fragrance market or for pharmaceutical applications [21]. They have a complex composition dominated by terpenoids, which are responsible for the flavor and antibacterial properties of EOs [22]. EOs also contain phenolic compounds, low molecular weight aliphatic hydrocarbons, aliphatic alcohols, aldehydes, ketones, acids, acyclic esters, or lactones [23]. In some cases, the antibacterial properties
are due to a synergistic effect obtained also with the contribution of other minor components. Some examples are *Salvia officinalis*, certain species of *Thymus* and *Oregano* [21].

Many essential oils were also studied for their antibacterial properties, especially in relation with food, and this trend is continuing due to the consumers’ interest for products’ safety with no synthetic additives used for preservation. The majority of essential oils and plant extracts are recognized as safe for commercial applications.

Many experimental studies have demonstrated that essential oils from oregano, thyme, basil, sage, rosemary, clove, coriander, caraway, fennel, nutmeg, pepper, cardamom, garlic, and onion exhibit antimicrobial activities against food-borne pathogenic bacteria [24].

The only problems are associated, on one hand, with the oils’ flavors, which are sometimes not tolerated by consumers’ taste, and on the other hand with their limit concentrations for the maximum antimicrobial effect desired, especially when these substances are applied directly on the food surface. For this reason, many essential oils are now incorporated in packaging materials in order to have a controlled release of the antimicrobial agents.

### 2.3.2 Antimicrobials of Animal Origin

There are numerous antimicrobial systems of animal origin, which act as defensive agents against a wide range of microorganisms. Very well documented is chitosan, which is also a biopolymer. Other antimicrobial agents of animal origin are lactoferrin, lactoperoxidase, lysosome, pleurocidin, defensins, histatins, and cathelicidins [24]. There are also lipids of animal origin with antimicrobial activity [23].

Chitosan, which is a deacetylated chitin derivative, is one of the most studied biopolymers which exhibit antimicrobial activity against a large number of microorganisms, being a nontoxic biopolymer [25]. It is already reported that the antibacterial activity of chitosan and chitosan oligomers is dependent on their molecular weight [26, 27]. Foster and Butt have demonstrated in a recent paper that only chitosan solutions have a strong bactericidal activity against a range of medically important bacteria, and one should note that the idea of antibacterial activity of chitosan films is an important misconception [28]. Yeasts and molds form the group that is the most sensitive to chitosan, and they are followed by Gram-positive and Gram-negative bacteria [24].

Various methods are used to prepare chitosan films, the most popular being the casting method [25]. Modern methods used to obtain chitosan composite films with other biopolymers are supercritical carbon dioxide treatment (chitosan/starch film), microwave treatment (chitosan/potato starch film) [29], and electronic beam irradiation [30]. Many essential oils and other antimicrobial substances were entrapped in chitosan or chitosan composite films used for food preservation.

Chitosan/cinnamon oil and chitosan/thyme oil films were used for preservation of refrigerated rainbow trout and chicken products [31]. The results were very promising, the coating film of chitosan with cinnamon oil being able to extend the shelf life during the refrigerated storage of fish samples. Antimicrobial films were
prepared by incorporating in chitosan matrix acetic and propionic acid, with or without addition of lauric acid or cinnamaldehyde, these films being used for preservation of meat products: bologna, regular cooked ham, or pastrami [32]. Antimicrobial edible films of chitosan containing garlic oil, potassium sorbate, and nisin were tested against food pathogenic bacteria. The most promising was the film with garlic oil, with the mention that it could be used only for food where the flavor of garlic oil is not a problem [33].

2.3.3 Antimicrobials Produced by Microorganisms
Bacteria are producing many compounds with antimicrobial properties in order to defend themselves against other bacteria by creating an unfavorable environment to other microorganisms. Bacteriocins can tolerate diverse treatments, such as boiling during pasteurization and sterilization of food, without losing much of their antimicrobial activity [24]. Bacteriocins are considered natural products because they are presented in fermented or non-fermented food from ancient times [34]. A large class of bacteriocins has been isolated from lactic acid bacteria, the most important members being nisin and pediocin, which are widely used for food preservation [34]. Nisin belongs to the lantibiotic class of bacteriocins, cationic and hydrophobic peptide [35]. Nisin has an antimicrobial activity directed primarily against Gram-positive bacteria and in particular against the spore forming ones [36–38]. Pediocins are effective against many strains of sublethally stressed Gram-positive and Gram-negative bacteria [35].

2.3.4 Nanoclays
Nanoclays are layers of silicate clay minerals composed of nanoplatelets with nanometric thickness. The most well-known of this class of minerals are montmorillonites (MMT), hectorite, and saponite. The incorporation of nanoclays in polymer composites has the aim to enhance polymer mechanical and barrier properties. Because many natural nanoclays are hydrophilic and cannot be used to obtain nanocomposites with hydrophobic polymers, they are chemically modified. In order to replace the inorganic cations with organic surfactants, which intercalate into the clay gallery, cation-exchange reactions are used [16]. Hong and Rhim (2008) have tested the antimicrobial activity against four representative pathogenic bacteria of three kinds of commercially available montmorillonite nanoclays. The first was a naturally occurring one (Cloisite Na⁺), and the others were organically modified (Cloisite 20A and Cloisite 30B). From the tested nanoclays, Cloisite 30B performed the best bactericidal effect against Gram-positive species and a remarkable bacteriostatic effect against Gram-negative organisms [39]. Cloisite® 10A, a modified montmorillonite with quaternary ammonium salt (organo-Mt), and Cloisite® Na⁺ as natural montmorillonite were tested against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 6538) for textile applications. From the two nanoclays tested, organo-Mt modified with ammonium quaternary salt showed better antimicrobial properties compared to natural montmorillonite [40]. Enhanced antimicrobial activity was measured in composites of nanoclays naturally and organically modified with different biopolymers and especially chitosan. Chitosan-based nanocomposite
films were prepared by Rhim et al. using an unmodified montmorillonite (Na-MMT) and an organically modified montmorillonite (Cloisite® 30B). Cloisite 30B-incorporated film showed a higher antimicrobial activity against *S. aureus* and *L. monocytogenes* than Na-MMT-incorporated film [41]. Other examples of antimicrobial activity of nanoclays combined with chitosan are given by Azeredo et al. [16]. Nanoclays combined with silver nanoparticles and copper exhibit also antimicrobial activity in powder form or incorporated in different polymer matrices (agar, zein, polycaprolactone, calcium alginate) [16, 42]. Nanoclays could have an indirect antimicrobial activity by controlling the diffusion or by enhancing the retention of other AM agents in polymer matrices [16].

3 Biopolymers Used in Food Packaging

The market of packaging materials is still being dominated by synthetic polymers that exhibit a great number of advantageous characteristics in terms of transparency, softness, mechanical resistance, heat sealability, and transfer properties. Being produced in very high quantities, they are generally available at low prices. Two are the main concerns for the future of plastics: one of them regards the depletion of natural oil resources (feedstock for synthetic polymers), and the second comes from the environmental pollution due to the lack of biodegradability of these materials. The alternative is represented by biodegradable polymers. These are investigated by many researchers, and some biopolymers are already commercially available. It is estimated that the production capacity of biobased polymers will reach nearly 12 million tons by 2020 [43].

Biopolymers could be natural ones, being extracted from renewable resources such as polysaccharides (cellulose, starch, chitosan, chitin, guar gum, alginate, carrageenan, pectin) and proteins (soy proteins, caseinates, gluten, zein) and synthetic ones which are obtained from oil-based monomers (polycaprolactones (PCL), poly(vinyl alcohol) (PVA), ethylene-vinyl alcohol (EVA) copolymers, polyesteramides (PEA)); there are also synthesized biopolymers from monomers obtained by fermentation (polylactic acid (PLA)) or obtained by microbial fermentation (polyhydroxyalkanoates (PHAs), bacterial cellulose, xanthan gum, and gellan). One of the problems associated with the use of biopolymers in food packaging applications is the difficulty of processing them in conventional equipment due to their poor mechanical properties. Some of the biopolymers are hydrophilic, and their low water resistance also limits their use as packaging materials. Generally speaking, their transport properties are sometimes inferior to those of synthetic polymers. Finally, their price is still high. Many of them are used in edible films and coating [44].

In order to obtain antimicrobial food pads, three biopolymers were used in the experimental part, these being bacterial cellulose (BC), carboxymethylcellulose (CMC), and xanthan gum (XG). For these biopolymers a brief presentation will be done to justify their use.
3.1 Bacterial Cellulose

Cellulose, which is the most abundant renewable organic material produced in the biosphere, serves as the dominant reinforcing phase in plant structures. The main sources for cellulose are wood or annual plants (e.g., cotton, hemp, linen, jute, flax, kenaf, ramie). In plants, cellulose is only a part of lignocellulosic material together with hemicellulose and lignin, and these components need to be separated. Cellulose is generally extracted by using one of the two methods, sulfate or by pre-hydrolysis kraft pulping (sulfate method). The kraft pulping method is the most popular and is responsible for around 80% of the world cellulose production. All the cellulose purification processes at industrial scale are high energy consumers, approximately 1000 kWh/ton, which represents still a high and expensive industrial activity [45]. Nowadays, green chemistry is also used to extract valuable wood components, but these attempts have only been tested at laboratory scale [46]. Even if wood and annual plants are recognized as the major source of cellulose, however, there are only a few microorganisms (bacteria, algae, tunicates, or fungi) which produce certain amounts of extracellular cellulose, known as microbial cellulose (MC) [47].

Cellulose produced by different bacteria strains was named bacterial cellulose (BC). Bacterial cellulose is one of the most promising biopolymers, being of great importance in the medical field as well as in many industrial areas. Bacterial cellulose is produced in different fermentation media, mainly by the acetic acid bacteria, which are members of the Acetobacteraceae family and particularly belong to the genera Komagataeibacter (classified first as Acetobacter and later as Gluconacetobacter genus) [45]. Bacterial cellulose is composed of (1→4)-β-glycosidic-linked glucose units. These linear glucan chains form highly regular intra- and intermolecular hydrogen bonds which confer to BC a high water affinity and other interesting properties. Bacterial cellulose (BC) is chemically identical to plant cellulose (PC) but possesses a different macromolecular structure and physical properties which give it special features, which are not encountered in cellulose obtained from plants. For example, both biopolymers have high crystallinity (usually in the range 40–60% for plant cellulose and above 70% for BC), and both are insoluble in water and other common solvents. Fibrils of BC are 100 times thinner than that of plant cellulose, making its structure more porous. The unique nanofibrillar structure of bacterial cellulose determines its potential application in the medical field, including potential scaffold for cartilage tissue engineering, wound dressing, and drug delivery systems [48–51]. For the present chapter, we have in view bacterial cellulose’s food applications. BC being pure cellulose, it could be used as dietary fiber and was recognized as safe (GRAS—generally recognized as safe) in 1992 in the USA [52]. A bacterial cellulose gel is consumed in the Philippines under the commercial name of nata as a low-calorie dessert. It is considered a dietary and healthy food. Its name could be completed with the carbon source of the fermentation medium: nata de coco is obtained from coconut, and nata de pina is obtained from pineapple [53]. BC could be also used to modify the rheology of food, being a thickening, gelling, stabilizing, emulsifying, and water-binding agent [52, 53]. BC was used in vegetarian meat in combination with...
Monascus extract to give the red color of the final product [52]. Food products containing BC could be consumed also for a low-cholesterol diet.

BC could as well be used in order to obtain food packaging materials in three ways: as pure material, as chemically modified, and, in most of the cases, as reinforcing material in composites with other polymers and biopolymers. In the first case, the BC’s use as food packaging is not economically feasible due to its high price. The possibility to use BC in biomedical applications like artificial skin, artificial blood vessels, artificial cornea, heart valve, wound dressing material, and artificial bone (especially as BC-hydroxyapatite composite) are more important, and the products are of high value [54]. The only feasible application of BC as pure material could be in document restoration. Santos et al. have tested lining papers with bacterial cellulose sheets [55]. The use of BC as reinforcing material may offer advantages for specific conservation treatments in comparison with other materials [55]. The conclusion of these studies was that BC may improve the physical properties of the damaged paper and could be a promising material for the restoration of paper documents [56]. The barrier properties of BC modified by controlled heterogeneous esterification with hexanoyl chloride were also studied for different applications of BC in the packaging industry [57]. BC-calcium carbonate composites were obtained in different conditions with possible applications for paper manufacturing [58, 59]. For meat, antimicrobial packaging BC containing nisin was used. The active BC films have produced the decline of L. monocytogenes populations on frankfurters, proving themselves as promising materials for antimicrobial meat packaging [60]. Functionalized films BC-lactoferrin were prepared as edible antimicrobial packaging, being used especially for fresh sausage as a model of meat products [61].

Due to its special properties, BC has been used in the preparation of a great number of composite materials with various applications. Having a high hydrophilicity, BC could be used to obtain composite materials with many biodegradable polymers. Among them, poly(vinyl alcohol) (PVA) is a very good candidate.

Composites PVA-BC with antimicrobial properties were prepared by our research team in order to be used as active food packaging materials. BC was used either as wet powder, or as wet fibrils, and was dispersed in PVA aqueous solutions. Sorbic acid was used as antimicrobial agent [4, 62, 63]. Sorbic acid release was studied from PVA-BC composites from monolayer films and from multilayer films. The multilayer films were obtained using also wet BC sheet as cover layers. The antimicrobial effect of PVA-BC composites containing also sorbic acid was tested against Escherichia coli K12-MG1655, which indicated that the new composites possess antimicrobial properties and could be used to obtain antimicrobial food packaging [64]. The antimicrobial activity was obtained also for composite PVA-BC containing sorbic acid, BC being dispersed as wet fibrils in PVA solutions. The designed film showed antibacterial effect against Escherichia coli and could be also considered a promising candidate for obtaining food antimicrobial packaging [62].

BC-silver composites that were reported especially for wound dressing applications could also be used for obtaining food packaging materials [65–68].
3.2 Carboxymethylcellulose

Carboxymethylcellulose (CMC) is a commercially available water-soluble cellulose ether of major interest in the hydrogel synthesis and for food packaging applications besides hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), and methylcellulose (MC). There are several available types of CMC based on particles’ size, degree of substitution, viscosity grades, and hydration characteristics. For general thickening applications, high viscosity grades are chosen for economic reasons, but in film applications, low-viscosity CMC solutions are preferred [69, 70]. CMC is one of the polysaccharides which have been used in edible films and coatings but also for encapsulation purposes. CMC is compatible with a wide range of other food ingredients, like proteins, sugars, starches, and other hydrocolloids [70]. Edible films starch/carboxymethylcellulose (CMC) were prepared by using a casting method. CMC/starch biocomposite films could replace starch films, since they have better physical and mechanical properties than starch films [71]. A hydrogel film containing CMC and polyvinylpyrrolidone was synthesized under controlled environmental conditions. The obtained films are transparent and flexible and show good mechanical properties. Being also biodegradable, they could be used as food packaging material [72]. Superabsorbent hydrogels containing CMC will be presented in another paragraph.

3.3 Xanthan

Xanthan gum is a water-soluble anionic polysaccharide produced by Xanthomonas campestris, being a well-known food hydrocolloid. Xanthan is nontoxic and for this reason has been approved by the United States Food and Drug Administration (FDA) as food additive. It is used in food industry as thickener and stabilizer. It has the property to control the rheology of water-based systems, and even at low concentrations, its solution has still a high viscosity. Its aqueous solutions exhibit pseudoplastic properties. Here is a brief enumeration of the roles it can play: as hydrocolloid it is used in bakery product production, as bodying agent in beverages and squashes, in dairy industry as stabilizer of many products, in dressings, for pet food production, syrups, and toppings obtaining [73]. Xanthan has also applications in pharmaceutical, cosmetic, gas, oil, and other industries. For the aim of this paper, we are interested in the films and hydrogels containing xanthan and which could be used in food packaging or as superabsorbent materials. Xanthan hydrogels could be obtained by chemical crosslinking using epichlorohydrin, glutaraldehyde, metabisulfite, citric acid, adipic acidic dihydrazide, or sodium trimetaphosphate to form hydrogels [74, 75]. Hydrogels containing xanthan could be obtained by chemical crosslinking using graft polymerization.

Physical crosslinking can also lead to obtain xanthan hydrogels by freeze thawing, ionotropic gelation, and polyelectrolyte complexation. A physical xanthan gel was obtained by a simple method through incorporation of montmorillonite particles at a critical concentration. The results have indicated that 2.0% w/w of
MTT concentration is sufficient to obtain a consolidated hydrogel [76]. Chitosan and xanthan gum, being polyelectrolyte with opposite charges, they could form polyelectrolyte complex gels [77, 78].

Hydrogels of xanthan crosslinked by esterification reaction at 165 °C, in the absence or in the presence of citric acid, were obtained and characterized. Their swelling behavior was investigated under different medium characteristics, as salt type and pH. Cross-linking of xanthan in the presence of citric acid forms a network with higher cross-linking density and low swelling degree [79, 80]. A superporous hydrogel was synthesized through chemical crosslinking by graft copolymerization of 2-hydroxyethyl methacrylate (HEMA) and acrylic acid (AA) onto xanthan gum (XG) by Gils et al. [81]. The prepared hydrogels have a high swelling degree and de-swelling ability, are biodegradable, and have a low content of residual monomers, being suitable for biomedical applications. Acrylamide was also used in a graft polymerization to synthesize a crosslinked hydrogel containing xanthan [82]. The measured properties of the obtained hydrogel recommend it as a pH and temperature-sensitive smart polymer. Another example of a graft copolymer is the one obtained from N-vinyl-2-pyrrolidone and xanthan gum [83].

Interpenetrating polymer network (IPN) hydrogel microspheres of XG and poly(vinyl alcohol) (PVA) were prepared for drug delivery. As model drug ciprofloxacin hydrochloride was used [84].

Organic-inorganic composites superabsorbent xanthan gum-g-poly(acrylic acid)/loess (XG-g-PAA/loess) in aqueous solution were also synthesized. A composite XG-g-PAA/loess containing 2 wt% of loess has a very high swelling ratio in distilled water (maximum 610 g/g) and only 54 g/g in NaCl solution [85].

### 4 Cellulose-Based Superabsorbent Hydrogels

Superabsorbent polymer (SAP) materials are hydrogels that can absorb and retain water or aqueous solutions up to thousand times of their own dry weight. Due to their excellent hydrophilic properties and high swelling rate, SAPs have been used in many applications, the most well-known being those in the medical area (especially as infant diapers and antibacterial materials) and also in the agricultural field to improve soil water retention and other soil properties. Furthermore, SAPs could be used also as delivery materials for drugs, fertilizers, or other active substances [86–90]. SAPs could be divided into two main classes: synthetic and natural. Most of the superabsorbent materials which are currently in use are produced from acrylic acid (AA) or its salts and from acrylamide (AM), using different polymerization techniques [91, 92]. These crosslinked polymers have a very high swelling degree (1000 g water/g SAP) but also present some drawbacks, because they are non-biodegradable and are obtained from petroleum.

For these reasons, there is a growing interest for developing new SAPs starting with biodegradable polymers such as cellulose and its derivatives, starch, chitin, and chitosan [93–95]. Cellulose and its derivatives (CMC, hydroxypropyl methylcellulose, methylcellulose, and hydroxyethyl cellulose) were used to prepare hydrogels
either using physical or chemical synthesis methods. The most frequently encoun-
tered physical methods for obtaining hydrogels are heating-cooling polymer solu-
tion, ionic interaction, complex coacervation, H-bonding, and freeze thawing, thoroughly described in many review papers \[96, 97\]. Not all the specified methods could be used to obtain hydrogels starting from cellulose and its derivatives, and not all the hydrogels obtained are superabsorbent materials. Some examples will be given below.

Aqueous solutions of methyl and hydroxypropyl methylcellulose were used to prepare reversible hydrogels. Gelation of these solutions is due to hydrophobic interaction between molecules containing methoxyl substitution. The degree of substitution of the cellulose ethers and the presence of salts in solution are influen-
ting the sol-gel transition temperature \[98, 99\]. Li (2002) has prepared also thermo-
reversible gels starting from aqueous solutions of methylcellulose, the parameters which were varied being methylcellulose concentration and temperature \[100\]. Thermo-gelation behavior of hydroxypropyl methylcellulose and hydroxyethyl methylcellulose aqueous solutions was investigated by rheology to determine the aggregation temperature and gelling temperature \[101, 102\]. An interesting idea was to prepare hydrogels of cellulose derivatives based on cryogenic treatment and UV irradiation. In this technique there were used semi-dilute aqueous solutions of different cellulose derivatives, containing photoinitiator, maintained at negative temperatures \((-20^\circ\text{C})\) and then crosslinked under UV irradiation. The main advan-
tages of these techniques are the simplicity and the extremely short irradiation time required for obtaining high-quality cryogels \[103\]. Not only cryogels of cellulose derivatives were prepared by using this method but also cryogels based on two biodegradable polymers, one of them being hydroxyethyl cellulose and the other being chitosan. \(\text{H}_2\text{O}_2\) and \(N,N'\)-methylenebisacrylamide were used as photoinitiator and, respectively, as crosslinking agent \[104\]. Cellulose/xanthan gum composite films and hydrogels were obtained through gelation with 1-butyl-3-methylimi-
dazolium chloride (BMIMCl), which is an ionic liquid. The cellulose/xanthan gum composite hydrogels were prepared by immersing the ion gels in water. Their water content is very high, more than 90\% \[105\]. Using a mixture between NaOH/urea as cellulose solvent, Chang et al. (2008) have prepared composite hydrogels cellulose/ poly(vinyl alcohol) by freeze/thaw, repeating the treatment with and without a chemical crosslinker (epichlorohydrin). Equilibrium swelling degree was higher for the chemical crosslinking samples than the physical hydrogels \[106\]. The sam-
ple prepared by repeating freezing/thawing cycles exhibit a dense structure between cellulose and PVA and in consequence have a high mechanical strength \[106\]. Thermally sensitive pH-dependent gel containing a cationic polysaccharide (chitosan) and CMC was obtained by physical methods. The polyelectrolyte solu-
tions formed by the two biopolymers remain liquid at physiological pH but turn into gel at body temperature. In the authors’ opinion, hydrophobic interactions seem to be the main driving force to form a chitosan gel in the presence of CMC, depending on the working temperature \[107\].

Chemical synthesis methods are widely used to fabricate cellulose and cellulose derivative-based superabsorbent hydrogels. Chemically crosslinkers such as
aldehyde (e.g., glutaraldehyde, adipic acid dihydrazide), epichlorohydrin, urea derivatives, carbodiimides, and multifunctional carboxylic acids are used for cellulose and its derivatives crosslinking [99]. The crosslinking reactions among the cellulose chains might take place not only in water solution but also in organic solvents. Solvents, such as N-methylmorpholine-N-oxide (NMMO), ionic liquids (ILs), and alkali/urea (or thiourea) aqueous systems, have been developed to dissolve cellulose, widening the opportunities for the preparation of cellulose hydrogels. These systems are reviewed by Chang and Zhang [108].

Many chemically crosslinker agents are toxic even at low concentrations, and for many applications this could be a serious problem [96, 99]. A green alternative is offered by polycarboxylic acids; in this case the mechanism of crosslinking is condensation reactions between hydroxyl groups of polysaccharides with carboxylic acids [96, 99]. A brief review will be presented by taking into account recently published research papers on this subject.

Cellulose/carboxymethylcellulose (CMC) superabsorbent hydrogels in NaOH/ urea aqueous system, using epichlorohydrin as crosslinker, were prepared. In this hydrogel, cellulose molecules act as the strong backbone of the network structure and CMC, which is highly hydrophilic and contributes to the high swelling ratio. The experimental results have proved that the equilibrium swelling ratio could be improved by changing the amount of CMC [93]. Hydrogels were also prepared starting from cellulose and sodium alginate solution and using epichlorohydrin as crosslinker, with the aim to obtain materials with large porous structure. The cellulose solution was prepared using 6 wt% NaOH/4 wt% urea/90 wt% water mixture. The maximum equilibrium swelling ratio which was attained was 253.7 g/g [109]. Cellulose/carboxymethylcellulose (CMC) hydrogels were synthesized using the same solvent mixture for cellulose (NaOH/water/urea) but a nontoxic crosslinker, polyethylene glycol diglycidyl ether (PEGDE). The highest swelling ratio obtained was 230 g/g. In saline solution, the swelling ratio diminished significantly, this being a limiting factor for future applications of the prepared hydrogels [110]. An interesting idea was to prepare superabsorbent hydrogels with antimicrobial properties and good mechanical strength, starting from quaternized cellulose (QC) and native cellulose in NaOH/urea aqueous solution and using epichlorohydrin as crosslinker. The properties of the obtained hydrogels depend very much on the contents of quaternary ammonium groups in hydrogel networks [111].

Chitin/CMC hydrogels were prepared using epichlorohydrin as crosslinker. For chitin solubilization there was used the same mixture as cellulose (8 wt% NaOH/4 wt % urea in water). High swelling ratios were obtained, confirming that the chitin/ CMC hydrogels could be considered as superabsorbent hydrogels. The hydrogel with the following composition: 20 g (3 w% chitin solution) and 80 g (3 w% CMC solution), was the most effective in distilled water, having a swelling ratio around 1200 g/g. The swelling degree is increasing with the increase content of CMC [112].

Highly absorbing cellulose-based hydrogels were synthesized by crosslinking carboxymethylcellulose and hydroxyethyl cellulose aqueous solution using divinylsulphone (DVS) or carbodiimide as crosslinking agents [113, 114]. For the samples crosslinked with DVS swelling, the measurement was done in water but
also in ionic solutions and at different pH values. For all the studied samples, an increase of the swelling ratio was measured with the increase of the pH of the external solution [113]. A superabsorbent material with agricultural uses was obtained starting from the same biopolymers carboxymethylcellulose and hydroxyethyl cellulose, citric acid being used as catalyst, and a carbodiimide as crosslinker. A pilot study has confirmed the possibility to use these hydrogels as water reservoir in the cultivation of a “cherry tomato” [115]. Hydroxypropyl methylcellulose (HPMC) was crosslinked with citric acid (CA) and sodium dihydrogenophosphate (NaH$_2$PO$_4$) being used as catalyst. The obtained films were tested for their moisture resistance in order to be used as packaging materials [116]. Two cellulose derivatives, namely, sodium carboxymethylcellulose (CMCNa) and hydroxyethyl cellulose (HEC), were used for hydrogel preparation using citric acid as crosslinker. Citric acid concentration was varied between 10% and 20% (w/w polymer). Hydrogels with high swelling degree were obtained with a reduced concentration of citric acid. For a hydrogel containing CMCNa/HEC weight ratio 3/1 mixture with 3.75% w/w CA, a swelling degree of 900 g/g was measured. The proposed reaction mechanism is an esterification one, being based on an anhydride intermediate formation. The obtained materials were destined to be used as superabsorbents in agriculture [117]. Not only citric acid was used as crosslinker but also malic and succinic acids. Hydrogels with antimicrobial properties were prepared using CMC as biopolymer and the aforementioned carboxylic acids as crosslinkers. As antimicrobial particles ZnO$_2$ were synthesized. The composite hydrogels have a high swelling degree and also a very good antibacterial activity against Gram-positive and Gram-negative bacteria [118].

Not only chemical crosslinkers can be used to obtain superabsorbent hydrogels but also physical treatments (i.e., electron-beam irradiation, gamma irradiation, microwave irradiation during polymerization) [92]. Ibrahim and Salmawi have used CMC and sodium alginate (SA) blends to prepare hydrogels using different gamma rays irradiation doses. The irradiation dose influences the gel fraction and the swelling degree. The obtained hydrogels have also antimicrobial activity [119]. The same biopolymers (CMC/SA) were used to prepare superabsorbents by gamma irradiation. The ability of these hydrogels to adsorb metal ions from wastewater was tested [120]. CMC and polyvinylpyrrolidone were crosslinked with gamma irradiation in order to prepare superabsorbent hydrogels with agricultural uses. As in the previous examples, hydrogels have a good swelling degree, which is greatly affected by their composition and absorbed dose [121]. Superabsorbent gels were prepared by gamma irradiation from aqueous mixtures of carboxymethylcellulose and starch by Fekete et al. [122]. Hydrogels containing 30% starch and prepared at 20 kGy irradiation dose showed the best swelling properties (water uptake of ~350 g water/g gel), especially in an environment with high electrolyte concentration.

Cellulose-based superabsorbents could be synthesized by grafting acrylic acid and other monomers to cellulose or its derivatives. As polymerization methods, the following have been used: aqueous solution polymerization, inverse-phase suspension polymerization, and microwave irradiation polymerization, largely described by Ma et al. [92].
Hydroxyethyl cellulose (HEC)/acrylic acid (AAc) copolymer gels with superabsorbent properties were synthesized from aqueous solutions by radiation-initiated crosslinking [123]. The HEC content was maintained as high as possible for a higher biodegradability of the final product. Effects of acrylic acid ratio, solute concentration, and absorbed dose upon hydrogel properties, and especially upon their swelling degree, were studied [123].

Cellulose and cellulose derivative-inorganic hybrid hydrogels are also in the attention of the researchers due to the fact that materials with high functionality could be developed by incorporation of inorganic particles in the polymer structure. Cellulose/acrylic polymer/inorganic particle superabsorbent composites were obtained using silicon dioxide (SiO₂) and titanium dioxide (TiO₂). Graft copolymerization of acrylic monomer was used as a method to prepare new superabsorbent composites using cellulose derivatives. For the composites incorporated with different cellulose derivatives, the swelling degree in distilled water and saline solution is the highest for those superabsorbents containing carboxymethylcellulose [124]. Wheat bran, which has a high content of natural cellulose, hemicelluloses, lignin, and protein, was modified by graft copolymerization of acrylic acid and various clays with the aim to prepare nano-hybrid organic-inorganic superabsorbents. The swelling degree is very much influenced by the type of clay used in the superabsorbent preparation. The new composites could be good candidates for agricultural and horticultural applications, their capacity of urea loading being also tested [125]. Graft polymerization of acrylic acid onto hydroxyethyl cellulose was performed in the presence of diatomite clay as nano-filler, N,N₀-methylenebisacrylamide as crosslinker, and ammonium persulfate as initiator. A maximum swelling ratio of 1174.85 g/g was measured in distilled water and 99.55 g/g in a saline solution (0.9 wt% NaCl) [126]. Not only clays but also graphene oxide was used as filler for incorporation in a superabsorbent obtained by graft polymerization of acrylic acid on carboxymethylcellulose backbone. The hybrid superabsorbents with graphene oxide have a higher swelling degree and an enhanced thermal stability in comparison with the copolymer without filler [127].

Interpenetrating polymer network is defined as a blend of two or more polymers in a network with at least one being synthesized and/or crosslinked in the presence of other, without any covalent bonds between them [128]. Many other polysaccharides or their derivatives have been used in the preparation of semi-IPN or IPN composite hydrogels [128]. Interpenetrating polymer network technology has been applied for synthesis of cellulose-based superabsorbents. Many examples of cellulose-based hydrogels which could be considered as interpenetrating polymer networks are given by Chang and Zhang [108].

5 Antimicrobial Food Pads

Moisture absorbers (MA) play a very important role for the extending shelf life of food, as the excess moisture is a major cause of spoilage. MA could be sheets, blankets, and pads [2]. They could be destined to fruit and vegetables but also for
meat, poultry, and fish products. Using MA, the food quality could be maintained by inhibiting microbial growth and avoiding the food degradation because of high water content. Even if the unsanitary juices are absorbed by MA, they could also generate undesirable odors or could lead to food spoilage. For this reason the incorporation of antimicrobial agents into a food pad could solve this problem. As classical desiccants used in the forms of sachets, the following could be mentioned: silica gel, calcium oxide, activated carbon, molecular sieves, and natural clays [129, 130]. They are used for moisture absorption for products like cheese, chips, nuts, candies, peanuts, and spices [2]. Nowadays the architecture of food pads is more complicated. Common absorbent pads include an intermediate layer formed by the absorbent material between two layers of microporous or non-woven polymer [130]. The upper and lower materials could be flexible thermoplastic films or coated cellulosic material. Different combinations of these materials are also in use, being largely described by Otoni et al. [131]. As moisture absorber, many materials like non-woven cellulosic pads, superabsorbent polymers, absorbent gels, and thermoplastic polymer fibers are used. Superabsorbent polymers which were tested as absorbing materials are polyacrylate salts, carboxymethylcellulose (CMC), and graft copolymers of starch [129]. The next step in the evolution of food pads was the incorporation of antimicrobial agents in their composition. Nanoparticles such as silver and cooper were already studied as antimicrobials for food packaging [131–134]. EOs are more promising than antimicrobial substances, being naturally obtained. Among the most tested EOs against food pathogens are *Allium sativum* essence oil, basil, bergamot, clove, cinnamon, coriander, eucalyptus, lemon, lemon-grass, oregano, rosemary, sage, thyme, and tea tree [21, 135, 136]. The synergism between different EOs and other substances has also been studied [135]. Combinations of plant extracts could be used also with the aim to minimize concentrations and consequently reduce sensory impact [137]. Very important is also the interaction between essential oils and food ingredients [137]. The use of bacteriophages to control pathogens is also a promising way to reduce microbial risk potential. Because the incorporation of these antimicrobial agents is still not very well-known, Gouvêa et al. have tested a mix of six bacteriophages in meat trays to be used for refrigerated foods [138]. Some commercially available moisture regulators which could be applied to meat products are presented by Ahmed et al. 2017 [130].

6 Synthesis of Carboxymethylcellulose, Xanthan Gum, and Bacterial Cellulose Hydrogels

6.1 Materials

All chemicals are commercially available, being of analytical grade (purchased from Sigma-Aldrich) and used without further purification. All solutions were prepared using high-quality deionized water. For antimicrobial activity measurements, were used three media. The first one is YEPD medium or yeast extract peptone dextrose, also often abbreviated as YPD medium, which is a complete medium for yeast.
growth. It contains yeast extract, peptone, double-distilled water, and glucose or dextrose. It was used as solid medium by including agar. The second one is PDA medium (potato dextrose agar – a specific medium for the fungi cultivation and estimation of mold population in food industry). For bacterial strains a third medium, Nutrient Agar, was used.

6.2 Hydrogel Preparation

Biocomposite hydrogels containing carboxymethylcellulose (CMC), xanthan gum (XG), and bacterial cellulose (BC) hydrogels were prepared by dissolving CMC and/or xanthan in deionized water together with a certain quantity of citric acid, in order to obtain different ratio between the two components. For the hydrogels containing also bacterial cellulose, this biopolymer was first grinded in wet state and then dispersed in the polymer solution containing CMC and/or CMC-xanthan. The solutions were homogenized with a stirrer at 200–300 rpm for half an hour. The obtained solutions were centrifuged for 5 min at 3600 rpm to remove air before casting. The solutions were poured into Petri dishes and dried at 50 °C for 16 h. The resulting dried films were used for extraction of the sol fraction using warm water (50 °C) under gentle magnetic stirring. The composition of the studied films is presented in Table 1.

6.3 Hydrogel Characterizations

The composite hydrogels were examined on a Jasco FT/IR6200 spectrometer (ABL & E-JASCO Romania) with Intron μ Infrared Microscope with ATR-1000-VZ objective. The spectra were the average of 50 scans recorded at a resolution of 4 cm⁻¹ in a range from 4000 to 500 cm⁻¹ with a TGS detector. For morphological observations, a scanning electron microscope FEI Quanta Inspect F scanning electron microscope (SEM) was used. All samples were gold coated prior to SEM examination. The thermal behavior of the hydrogels was tested using thermogravimetric analysis on a thermal analyzer (DTG-60-Shimadzu). The operating conditions were temperature range of 20–1000 °C, with a heating rate of 10 °C/min, and air flow rate of 50 mL/min.

Swelling degree was determined as a function of time in deionized water. The average mass of the dried hydrogels was approximately 500 mg. The dried weighed samples were immersed in excess deionized water at ambient temperature and were weighed at different time intervals. The free water was removed using a paper towel to be sure that the net weight of each sample will be measured. The swelling degree (Q (g/g)) was calculated using Eq. 1.
where $M_s$ and $M_d$ are the weight of the swollen and, respectively, of the dried film. Swelling kinetics was also measured using the same procedure, but the samples were weighed at predetermined time intervals.

Gel content was measured at room temperature. The pre-weighed hydrogel films were immersed in distilled water for 24 h, after which the samples were removed and dried at constant weight. Gel fraction (% w/w) was calculated using Eq. 2.

\[
\text{Gel fraction} = \frac{W_2}{W_1} \times 100
\]

where $W_1$ is the sample weight before extraction and $W_2$ is the dried sample weight after extraction.

### 6.4 Results and Discussions

#### 6.4.1 Gel Fraction

In Fig. 1 are presented the gel fractions for all the studied samples. As one can see, the values of the gel fractions are between 70% and 90%. The lowest gel fractions were obtained for the samples without XG (the films P1 and P2), and the highest values were obtained for a ratio CMC/XG 1:3 (w/w) (the films P5 and P6).

#### 6.4.2 Swelling Properties of the Hydrogels

Swelling degree of the studied samples is presented in Fig. 2. One could observe large differences between the samples, in a good correlation with their composition. The samples which contain only CMC and citric acid (CA) in a ratio CMC/CA (4:1 w/w) have low swelling degrees. The BC presence enhances the swelling, but not very much. The swelling degree is 4.61 g/g for sample P2 in comparison with 3.73 g/g for sample P1. When XG is introduced in the hydrogel composition, the swelling degree is increasing with the increase of xanthan content. The highest

<table>
<thead>
<tr>
<th>Table 1 Hydrogel composition</th>
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<tbody>
<tr>
<td>Composition (g)</td>
</tr>
<tr>
<td>CMC</td>
</tr>
<tr>
<td>Xanthan gum</td>
</tr>
<tr>
<td>BC (wet gel)</td>
</tr>
<tr>
<td>Citric acid</td>
</tr>
</tbody>
</table>
Fig. 1 Gel fractions for the studied samples

Fig. 2 Swelling degree for the studied samples
swelling degree was obtained for the samples having a ratio between CMC/XG (1:6 w/w), CMC/CA (5:4 w/w), and XG/CA (7.5:1 w/w). BC presence, even in a very low quantity if we report to its dry mass, has also influenced swelling degree by increasing it. Having in mind the aim of this work, to propose new biodegradable antimicrobial pads for food preservation, we have studied only the properties of the samples P7 and P8.

In Fig. 3 SEM images of the studied samples P7 and P8 are presented. A good compatibility between components could be observed. In Fig. 3b the presence of BC, even if it is in a low quantity, is observed as microfibrils dispersed in the hydrogel structure.

In Fig. 4 are presented FTIR spectra of the samples P7 and P8 which were tested to be used in food pad applications. The two spectra are practically identical, and the most of the absorption bands could be attributed to XG, which is an expected result, due to the hydrogel compositions (77% (P7) and 76% (P8) XG). The peak at 3331 cm\(^{-1}\) could be assigned to O–H stretching, and it is common for all the polysaccharides which are the components of the hydrogel (CMC for P7 and CMC and BC for P8). The same assumption could be made about the peaks at 1151 cm\(^{-1}\) (C–O–C asymmetric stretching vibration for glycosidic ring) and 1054 cm\(^{-1}\) (C–O–C pyranose ring skeletal vibrations) that are also common to all polysaccharides. The peaks between 1600–1640 and 1400–1450 cm\(^{-1}\) could be assigned to symmetric and asymmetric vibrations of ionized –COO\(^{-}\) group. Bands between 1710 and 1730 cm\(^{-1}\) correspond to axial deformation of C=O ester, acid carboxylic, aldehydes, and ketones [139]. Unfortunately the presence of ester bonds could not be identified at 1730 cm\(^{-1}\) in the presence of citric acid, even if the mechanism of the crosslinking reaction between citric acid and the polysaccharides has been reported to take place by ester linkages formation [79].

The XRD patterns of the samples P7 and P8 are displayed in Fig. 5. The large and most intense band centered at about 20° was assigned to carboxymethylcellulose phase, which is the more crystalline in comparison with xanthan [140]. This band
could also conceal the contribution of xanthan phase, which is the majority phase; however, its presence in crystalline form is slightly signalled through the flattened and broad band places between $30^\circ$ and $50^\circ$ [80]. Xanthan is considered to be almost

![Fig. 4 FTIR spectra of the samples: (a) P7 and (b) P8](image1)

![Fig. 5 X-ray diffraction patterns of the samples P7 and P8](image2)
similar to that of a typical semicrystalline amorphous material [141]. The sharp peak centered at approximately 29°C could be related to the use of citric acid for the preparation of the composites [142]. Unfortunately, the addition of bacterial cellulose in the sample P8 is not evidenced by this characterization technique due to the low content and pronounced contribution of carboxymethylcellulose.

6.4.3 Thermal Analysis

Figure 6 presents the corresponding TGA curves in a comparative approach. Generally, the thermal effects emerged for the investigated samples overlap on the entire temperature range, with some differences in terms of intensity. Three main weight loss steps can be identified on the derivative curves, associated with exothermic effects, showing the successive burning of the constituent polymers: citric acid at around 220 °C, xanthan at approximately 290 °C, and carboxymethylcellulose in the range 400–500 °C [143–145]. Considering the temperature of 560 °C, sample SP7 exhibits a weight loss of 91.42% while SP8 of 89.40%, a fact that indicates a slightly improved thermal behavior for the biocellulose containing material. Moreover, the weight loss occurred in the 170–240 °C temperature range is more pronounced in the case of P8, phenomenon that can be correlated with either the compositional changes or the existence of local inhomogeneity of the composite.
6.5 Adsorbent Pads with Antimicrobial Properties

In order to realize biodegradable adsorbent pads with antimicrobial properties, two problems must be solved. The first one is to cover the adsorbent material with an upper and lower layer. In some cases these layers are constituted by impermeable thermoplastic material or by cellulosic materials [131]. More materials were tested as upper and lower layers for the sample P7 and P8. The first coating material was chosen based on previous experience as PVA/BC mixture [146].

6.5.1 Preparation of Double-Layer Composite Films

The above obtained composite films (P7 and P8) were coated with PVA/BC mixture. The monolayer films (P7 and P8) were dipped in the PVA/BC mixture and then carefully dried at 50 °C in order to obtain uniform coated layers. The coating layer was obtained by dissolving PVA in water under magnetic stirring at 90 °C for 4 h. In the PVA solution, were dispersed various amounts of wet ground BC fibrils corresponding to different ratios between the two biopolymers PVA/BC (5:0.2) and (10:0.2) (g/g expressed as dry mass). The covered films were noted P7a and P8a when the cover layer was composed of PVA/BC (5:0.2) g/g dry polymer and P7b and P8b when the cover layer was composed of PVA/BC (10:0.2) g/g dry polymer.

Fig. 7  Swelling degree for the uncovered (P7 and P8) and double-layer films (P7a, b and P8a, b)
6.5.2 Swelling Properties of the Double-Layer Composite Films

In Fig. 7 are depicted the results obtained for the swelling degree of the covered samples in comparison with the uncovered ones. Even if a decrease of the swelling degree was expected, the measured decrease was a drastic one, and the new values were not acceptable for a food pads system. Under these conditions we have decided to test other materials which are biodegradable and which could act as cover layers without such decrease of swelling degree. As cover materials for the samples P7 and P8, were used gauze (P7c and P8c), flat cotton (P7d and P8d), and cellulosic composite paper with polyester (P7e and P8e).

In Fig. 8 are depicted the results obtained for the swelling degree of the new composite samples. In comparison with the uncovered samples, the swelling degree is also decreasing, but it is still maintaining at acceptable values for the samples P7e and P8e. So, we recommend as cover material the cellulosic composite paper with polyester.

6.5.3 Antimicrobial Agent Release

The samples P7 and P8 were also prepared adding to the initial compositions of two antimicrobial agents, a commercial one (potassium sorbate) and a natural one (thyme essential oil, ThEO). The release experiments of potassium sorbate were carried out cutting film disks with 2.5 cm diameter which were introduced in a backer containing 100 mL of distilled water. The diffusion medium was homogenized using a magnetic stirrer. Sample of solutions (0.1 mL) were removed at the predefined time.

Fig. 8 Swelling degree for the uncovered (P7 and P8) and double-layer films covered with different materials
intervals. The concentration of the potassium sorbate was measured by a UV/VIS spectrophotometer (Cintra 6-GBC Scientific-Australia) at 254 nm, using a previous performed standard curve.

In Fig. 9 are depicted the release profiles for the studied samples. One could observe that the quantity of sorbate released is higher for the sample P8.

### 6.5.4 Antimicrobial Activity

As the antimicrobial activity of potassium sorbate is well documented, we have tested the antimicrobial activity of the superabsorbent film impregnated with thyme essential oil (ThEO). For the antimicrobial activity, were tested samples impregnated with ThEO in two ways: (1) a determined quantity of ThEO was poured in the film casting solution and dried at 50 °C for 16 h in an oven (code samples P7d, P8d), and (2) dried films without any microbial agent were impregnated on the surface with ThEO in concentration of 0.07, 0.14, and 0.21 μL/mm² (code samples P7f, P8f). Four microbial strains: *Escherichia coli*, *Bacillus subtilis*, *Candida utilis* (*Torula*), and *Penicillium hirsutum*, were tested. The bacterial strains were cultivated on nutrient agar medium. For the yeast strain, were used YPD medium and for the mold strain the PDA medium. The method used to evidence the antimicrobial activity of superabsorbent films impregnated with ThEO was the disk diffusion method. The Petri dishes with the culture media specific to every strain were inoculated with 100 μL cellular suspension with the concentration of 1 in McFarland standards

![Fig. 9 In vitro cumulative release profiles of sodium sorbate: (■) sample P7 and (▲) sample P8](image-url)
(equivalent of approximately $4 \cdot 10^6$ cfu/mL for bacterial strains and approximately $7 \cdot 10^5$ cfu/mL for fungi strains). On the surface of the inoculated media, were placed the disks of superabsorbent film impregnated with ThEO, with diameter of 6 mm. The samples were incubated at specific temperature of each strain: *E. coli* at 37 °C, *B. subtilis* and *C. utilis* at 30 °C, and *P. hirsutum* at 20 °C for 48 h, and after that were kept at low temperature (4 °C). The results obtained for the samples impregnated in two different ways are presented in the images below. In Fig. 10 are presented the control samples for the superabsorbent films P7 and P8 studied, without antimicrobial ThEOs impregnations. The presence of the control samples didn’t affect the microorganisms’ growth.

Figures 11 and 12 show the fact that the samples impregnated on the surface with ThEO (P7f, P8f) completely inhibited the microorganisms’ growth.

Fig. 10 The control samples against all microorganisms studied for P7 (a) and P8 (b) superabsorbent films

Fig. 11 Samples of P7f (a) and P8f (b) after 2 weeks of antimicrobial activity against three studied microorganisms. (0, ThEOs control sample; 1, 0.07 μL/mm² ThEOs; 2, 0.14 μL/mm² ThEOs; and 3, 0.21 μL/mm² ThEOs)
Superabsorbent films P7 and P8 impregnated with ThEO during casting solution preparation and then dried present a distinguishable inhibition zone, but also, the concentration of antimicrobial essential oil used exhibits the minimum inhibitory concentration (MIC), with purpose to ensure a long shelf life of the food product.

**Fig. 12** Samples of P7f (a) and P8f (b) after 2 weeks of antimicrobial activity against *P. hirsutum*. (0, ThEOs control sample; 1, 0.07 μL/mm² ThEOs; 2, 0.14 μL/mm² ThEOs; and 3, 0.21 μL/mm² ThEOs)

**Fig. 13** Antimicrobial activity for samples (a) P7d and (b) P8d on *Bacillus subtilis* and *Escherichia coli*, after 2 weeks at 4 °C.
In Figs. 13 and 14, are presented the antimicrobial activities for the samples P7d and P8d, against *Bacillus subtilis* and *Escherichia coli* (Fig. 13) and against *Candida utilis* (Torula) and *Penicillium hirsutum* (Fig. 14). The inhibition zones (IZ) were measured around the superabsorbent disks after 24 h. After 48 h from inoculation, the samples were stored in the refrigerator and kept for 2 weeks. The values of the

![Image](image_url)

**Fig. 14** Antimicrobial activity for samples (a) P7d and (b) P8d on *Candida utilis* (Torula) and *Penicillium hirsutum*, after 2 weeks at 4 °C

**Table 2** The IZ recorded around the superabsorbent films containing ThEO, after 24 h of incubation at specific temperature of the selected microorganisms, using the disk diffusion method

<table>
<thead>
<tr>
<th>Strain</th>
<th>Film code</th>
<th>IZ, mm</th>
</tr>
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<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>P7d</td>
<td>10–15</td>
</tr>
<tr>
<td></td>
<td>P8d</td>
<td>&gt;20</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>P7d</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>P8d</td>
<td>15</td>
</tr>
<tr>
<td><em>Candida utilis</em></td>
<td>P7d</td>
<td>5–8</td>
</tr>
<tr>
<td></td>
<td>P8d</td>
<td>&gt;20</td>
</tr>
<tr>
<td><em>Penicillium hirsutum</em></td>
<td>P7d</td>
<td>Negative results</td>
</tr>
<tr>
<td></td>
<td>P8d</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>
inhibition zones after 2 weeks were also measured. In all studied cases, the film samples without ThEO didn’t present antimicrobial activity.

The results recorded after 24 h of incubation are presented in Table 2, and the results recorded after 2 weeks at 4 °C are presented in Table 3.

The results concerning the antimicrobial activity show that the concentration of thyme used for the two methods of impregnation was higher than MIC (estimated at 6 ppm for bacterial strains and yeast and 60 ppm for molds).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Film code</th>
<th>IZ, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>P7_θ</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>P8_θ</td>
<td>&gt;20</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>P7_θ</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>P8_θ</td>
<td>10</td>
</tr>
<tr>
<td><em>Candida utilis</em></td>
<td>P7_θ</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>P8_θ</td>
<td>&gt;20</td>
</tr>
<tr>
<td><em>Penicillium hirsutum</em></td>
<td>P7_θ</td>
<td>&gt;20</td>
</tr>
<tr>
<td></td>
<td>P8_θ</td>
<td>&gt;20</td>
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To extend the shelf life of food and maintain its nutritional properties and sensory characteristics is a challenge for food industry. But it is not the only goal as, at the same time, the packaging materials must be recyclable and biodegradable in order to minimize the environmental impact. In the present, non-biodegradable synthetic polymers are the most used as packaging materials having many useful properties. The first part of this chapter tries to answer to the two challenges of the food industry. Antimicrobial active packaging is proposed, among other solutions, to extend the shelf life of food, and a brief overview of the most encountered antimicrobial agents was made. The attention was focused especially on the application of natural antimicrobial compounds of different origins, emphasizing on essential oils and other plant extracts. To reduce environmental pollution with non-biodegradable plastics, the solution of the biopolymer composites is proposed, out of which cellulose and its derivatives are very good candidates. There are a series of difficulties which must be overcome because biopolymers do not have all the properties of synthetic polymers, and at the same time, they are more expensive. A lot of steps were already made in this direction, and the researchers have studied many possibilities to overcome these difficulties. The recent papers published in this field are a good proof in this respect. The overview was also oriented through food pads, antimicrobial moisture regulators being a solution for food spoilage. In the architecture of a food pad, the superabsorbent material plays a key role, and of this reason superabsorbents containing cellulose and its derivatives were briefly presented. In
the second part of the chapter, experimental data are presented on obtaining and characterization of a new antimicrobial superabsorbent containing xanthan, carboxymethylcellulose, and bacterial cellulose.

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