#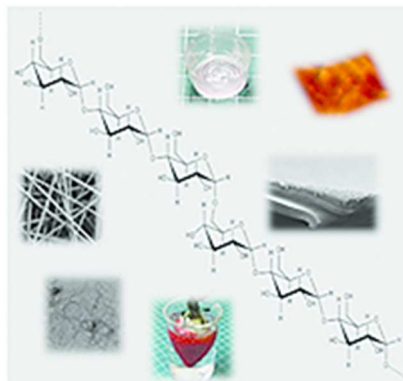 Pullulan-based films and coatings for food packaging: present applications, emerging opportunities, and future challenges

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Pullulan-based films and coatings for food packaging: present applications, emerging opportunities, and future challenges

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ABSTRACT

New societal challenges will impact our lifestyle in the coming years. At the moment, we are in the middle of a "green" revolution, stemming from societal and industrial demands for a lower environmental impact, cost effectiveness, and high-performance goods and services. Like many other sectors, food packaging is moving to new technologies; the use of biopolymers is one of the most promising strategies toward an optimized use of traditional packaging materials (e.g., oil-based plastics) without impairing goal of extending shelf life. Among other food packaging materials, pullulan is attracting much attention due to its unique features. The goal of this review is to provide an overview of current and emerging applications of pullulan within the food packaging sector. In particular, the functional properties of interest for the food packaging industry will be discussed in light of the physicochemical attributes of this exopolysaccharide. Future challenges that may dictate the successful penetration of pullulan in the food packaging market are also outlined.

KEYWORDS: Biopolymers and renewable polymers; coatings; film; packaging; nanostructured polymers; surfaces and interfaces

INTRODUCTION

A number of biopolymers are currently used in many different areas, such as in the food, pharmaceutical, and biomedical fields, due to their unique physicochemical properties, especially when compared to their oil-derived counterparts (e.g., plastics). Applications include their use as emulsifiers, stabilizers, binders, gelling agents, coagulants, lubricants, film formers, thickening agents, and suspending agents, just to provide a few examples.[1]

Pullulan is one of the biopolymers that have attracted much attention over recent years due to its peculiar characteristics. This non-ionic exopolysaccharide is obtained from the fermentation medium of the fungus-like yeast *Aureobasidium pullulans* (originally called *Pullularia pullulans*) under limiting conditions (e.g., nitrogen), with media composition and culture conditions highly affecting the final yield.[2] The production of pullulan by *A. pullulans* was first discovered by Bauer in 1938,[3] although isolation and characterization of pullulan were described by Bernier 20 years later.[4] The basic structure of pullulan was first provided by Wallenfels, Bender, Keilich, and Bechtler, who also coined the name "pullulan".[5,6] Large-scale production of pullulan was started in 1976 by Hayashibara Company Limited (Okayama, Japan); pullulan films from the same company appeared on the market in 1982.[7] Hayashibara is still the leading commercial producer of pullulan worldwide, selling food grade (designated as PF) and deionized (PI) products with a mean molecular weight of 100,000 (PI-10 and PF-10) or 200,000 (PI-20 and PF-20).[7]

Pullulan is generally marketed as a white to off-white dry powder.[1] It is non-toxic, non-mutagenic, non-carcinogenic, and edible.[8] It is tasteless, odorless, and highly soluble in both cold and hot water and in dilute alkali, though it is insoluble in alcohol and other organic solvents

except dimethylsulfoxide and formamide.[1] It has a considerable mechanical strength and other functional properties such as adhesiveness, film and fiber formability, and enzymatically mediated degradability, though it is not attacked by the digestive enzymes of the human gut.[7, 9] Other properties of this exopolysaccharide concern the stability of its aqueous solutions over a broad range of pH, the low viscosity in comparison with other polysaccharides, the inability to form gels, and the good oxygen barrier properties of films and coatings derivatives.[10] It has also been reported that pullulan inhibits fungal growth in foods.[11] As a biodegradable and biocompatible biopolymer, it has achieved wide regulatory acceptance with its proven safety record. In the United States, pullulan has "generally regarded as safe" status.[12] Due to its peculiar characteristics, pullulan is extensively used in different sectors, the three main realms of application pertaining to the pharmaceutical, biomedical, and food fields.

Pharmaceutical applications of pullulan mainly involve the manufacturing of hard and soft capsules, where pullulan is used instead of gelatin to meet a variety of cultural and dietary requirements, including those of vegetarians, diabetics, and patients with restricted diets.[13] More recent use of pullulan in the pharmaceutical field has involved the development of microspheres as carrier for drug delivery. It has been pointed out that pullulan confers stability, biocompatibility, and biodegradability to microspheres.[14,15]

In the biomedical field, pullulan has been proven to be effective in gene delivery, targeted drug therapy, tissue engineering, wound healing, and in diagnostic applications such as vascular compartment imaging and perfusion, receptor, and lymph-node target-specific imaging.[1] Pullulan was also explored as a potential blood-plasma expander to replace dextrans.[16]

In the area of the food industry, early applications of pullulan involved its use as a thickening, stabilizing, texturizing, and gelling agent, providing products with good sensory

properties, extended shelf life, and easier processing.[17] Later on, pullulan found application as filler for low-calorie food and beverages.[18] Previous works also claimed the potential use of dietary pullulan as prebiotic, promoting the growth of beneficial bifidobacteria.[19]

Pullulan is also being used in the cosmetics (lotions, shampoos, facial masques) and oral care (toothpaste, denture adhesive) sectors. A film-based oral care product containing pullulan is commercialized in many countries under the brand name Listerine.[20] Minor uses of pullulan include photographic, lithographic, optic, and electronic applications, and uses in chromatography as a molecular weight standard have also been reported.[13,19,21,22] More recently, pullulan has also been exploited for the production of microspheres for the removal of anionic dyes[23] and to generate novel biodegradable flocculating agents.[24]

The growing interest in this exopolysaccaride is demonstrated by the relatively large number of reviews and book chapters about it in the last 15 years[1,2,7,9–11,13,19,20,25–30] as well as by the amount of patent applications (from 2000 to 2007, ten new inventions related to pullulan have been reported).[20] All of these publications primarily focused on the biosynthesis/production of pullulan, its structural characteristics, and its applications in the pharmaceutical, biomedical, and food sectors. However, none of them reviewed in detail the applications of pullulan in the food packaging sector, which is actually an emerging area of interest.

This review has been conceived as guidance for both academics and industrial operators involved at different levels with the manipulation of this fascinating biopolymer for specifically intended food packaging applications. The main physicochemical properties of pullulan are discussed in a first section in order to better introduce the reader to the second part of this review, which is dedicated to the applications of this exopolysaccharide. Current uses are addressed first, and then the most recent and emerging applications are described. In the last part

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of this review, we outline the potential applications of pullulan, with special emphasis of those shortcomings that hinder its widespread use in the food packaging sector.

CHEMICAL STRUCTURE AND PHYSICAL PROPERTIES

Although the chemical structure of pullulan has been thoroughly reviewed in previous works,[9,19,20] the most important physicochemical features are recalled hereinafter to emphasize the structure–properties relationship of pullulan derivatives currently used or with potential application in the food packaging sector.

The chemical formula of pullulan has been suggested to be $(C_6H_{10}O_5)_n$.[20] Its chemical structure can be viewed as a maltotriose trimer given by the succession of α-(1→6)-linked (1→4)-α-D-triglucosides.[19,27] As discovered by Wallenfels in 1961,[6] the enzyme pullulanase specifically hydrolyzes the α-(1→6)-linkages in pullulan yielding quantitatively the oligomers $(G3)_n$, where G3 represents the α-(1→4)-linked repeating trimer maltotriose, and n is the number of trimeric repeating units in the oligomer (Figure 1). Maltotetraose subunits can also be found on the pullulan backbone to a maximum extent of ~ 7%, even though they do not affect the overall physicochemical properties of the polysaccharide.[31,32] Pullulan structure was unambiguously described by Arnosti and Repeta in 1995 through proton NMR spectroscopy.[33] The same authors revealed the occurrence of the α-(1→6)-linkage between every third glucose ring by ^{13}C NMR spectra. The co-existence of α-(1→4)- and α-(1→6)-glycosidic linkages in the pullulan structure was also established by the appearance of a band at $\nu = 935$ cm^{-1} in a typical IR spectrum.[34]

The regular occurrence of α-(1→6)-linkages in pullulan interrupts what would otherwise be a linear amylose chain.[19] Due to the alternation of α-(1→4)- and α-(1→6)- linkages in a single

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compound, pullulan structure is often seen as an intermediate between amylose and dextran structures. That is also why pullulan is much more water-soluble than amylose (which has only α-(1→4)-linkages).[13] Aside from the high solubility in water, this unique linkage pattern is also responsible for the high structural flexibility of pullulan. However, the segmental mobility of the pullulan backbone is not uniform, with the regions of increased mobility centered on the α-(1→6)-linkages.[35,36] This peculiar structure is also reflected in the lack of crystalline regions within the polymer: pullulan has a completely amorphous organization, as experimentally confirmed by recent studies.[37,38] The conformation of pullulan in aqueous medium is also influenced by the simultaneous presence of α-(1→4)- and α-(1→6)- linkages. C_1-O-$CH_2(C_6)$ linkage α-(1→6) is more flexible than C_1-O-C_4 linkage in which C_1 and C_4 belong to glucopyranose rings. Eventually, this leads to a random coil conformation of pullulan backbone in aqueous solutions, which in turn yields a bundled molecular arrangement (Figure 2a).[39,40]

From a practical point of view, high flexibility and lack of crystallinity provide pullulan with the capacity to form thin layers, electrospun nanofibers, nanoparticles, flexible coatings, stand-alone films, and three-dimensional objects (Figures 2b–g). These distinct features are not exhibited by other polysaccharides, making pullulan very similar to certain synthetic polymers derived from petroleum [e.g., poly(vinyl alcohol)–PVOH and nylon–PA 6,6)].[19] Other unique properties of pullulan, which will be discussed shortly, have been disclosed only recently.

CURRENT APPLICATIONS

Pullulan-based coatings on foods

Early attempts to employ pullulan in the food packaging industry lagged behind the established use as a food additive (e.g., thickening agent, binder, stabilizer), the first works dating back to

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the beginning of the '90s. At that time, it was understood that great benefits would have arisen from certain peculiar properties of pullulan, such as its high water solubility and the barrier property against oxygen and carbon dioxide. At the beginning, water-soluble edible films of pullulan were proposed as edible pouches for premeasured portions that could be gradually dissolved in water or in hot food.[41] However, the first massive application of pullulan in food packaging can be considered its use as an edible coating, i.e., a relatively thin layer of material applied and formed directly on the surface of the food product, which can be eaten along with the product.[42]

Edible coatings

The use of edible coatings to protect foods has long been known. Edible coatings can help prevent the deterioration of foods by retarding dehydration, suppressing respiration, improving textural quality, allowing the retention of volatile flavor compounds, and reducing microbial growth.[43] Due to this potential, the interest in the development and use of edible coatings to prolong shelf life and improve quality of fresh, frozen, and formulated food products has rapidly increased over the last two decades.[44] Pullulan edible coatings, in particular, were primarily suggested for fresh and minimally processed fruits and vegetables. The use of edible coatings for these kinds of food products stemmed from the need for high quality and minimal processing and storage technologies. Quality enhancement and shelf life extension can be thus achieved by properly selecting a film-forming biopolymer that allows the controlling of exchanges of moisture, gases (e.g., oxygen, carbon dioxide, ethylene), and aromas between food and environment.[45] Owing to its inherent hydrophilic nature, pullulan, as with most polysaccharides, can be advantageously used as a coating for the quality preservation of fruits and vegetables

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more as a gas barrier rather than retarding water loss. More specifically, the ability to reduce O_2

and increase CO_2 (due to the selective permeability to these gases) in internal atmospheres of

coated fruits and vegetables reduces respiration rates, thereby extending the shelf life of fresh

products in a similar way to modified/controlled atmosphere storage.[44] Compared with other

polysaccharides, pullulan offers additional benefits, such as high gloss and transparency, heat

sealability, and high capability to be mixed with other biopolymers, in addition to its derivatives

being tasteless and colorless.[46]

Pullulan coatings were successfully used to extend the shelf life of freshly harvested non-

climacteric and climacteric fruits, namely strawberries and kiwifruits, respectively. According to

the authors, the shelf life extension goal was achieved thanks to the reduced respiration rates of

the fruits ensuing from changes in the internal atmosphere (i.e., modification of the gases

composition).[44] Edible coatings made of pullulan were also used to extend the shelf life of whole

apples (Malinova and Champion cultivars).[47] Final results showed that pullulan coatings were

able to reduce the weight loss of fruits stored at 4 °C and 22 °C, which was attributed to the

physical barrier represented by the coating to the moisture migration. Pullulan coatings were also

laid on fresh-cut apple slices (Fuji cultivar) during hypothermia storage. The edible layer allowed

reducing the softening process of apple tissues as well as the weight loss and the respiration

rate.[48] Finally, pullulan was also used in the form of edible coatings on refrigerated white

asparagus spears.[49] As concluded by the authors, the edible coating had a beneficial impact on

white asparagus spears' quality retention during refrigerated storage by retarding their weight

loss and the purple color formation on the surface of the tips, and by keeping the spears at high

quality levels.

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To overcome some intrinsic limitations that may affect specific applications, pullulan was also used in combination with other biopolymers. The resulting coatings, due to both chemical and/or physical interaction between phases, exhibit improved properties pertaining to both the individual matrices and synergistic effects. Table 1 summarizes the most relevant examples of pullulan-based films and coatings with potential applications in the food packaging sector. In recent years, the properties of pullulan films have been investigated after the addition of nano-sized entities such as starch nanocrystals[37] and nanofibrillated cellulose.[64,65] The rationale behind these attempts was to exploit the synergies between the biopolymer matrix and the nanofillers, especially in terms of interface effects. Final bionanocomposites indeed showed remarkable improvement in terms of mechanical, thermal, and water absorption properties.

"Active" films and coatings

In the last two decades one of the most innovative developments in the area of food packaging is the "active packaging".[66] Active packaging includes systems capable of interacting with the food and/or the environment with the goal of enhancing food quality, safety, and convenience.[67] Examples of active packaging technologies include absorbing systems and releasing systems. The first group includes oxygen and carbon dioxide scavengers, humidity absorbers, exuded absorbers, and so on. However, the majority of research studies on active packaging for food and beverage applications mainly focus on the development of antimicrobial and antioxidant systems, which fall into the second group.

Because edible coatings can be intended as the primary packaging that is wrapping the food, they can take on all those "active" features that generally pertain to commonly used oil-based plastic films. Actually, the capability of biopolymers to "sense" external stimuli acting as

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triggers (e.g., temperature, pH, relative humidity variations) makes edible coatings more suitable than plastics from petroleum for the development of the second generation of active packaging, namely controlled release packaging (CRP).[68] The term "CRP" refers to packaging systems designed to deliberately control the release of active compounds at rates suitable for a wide range of food products and specific degradation reactions.[69,70]

In recent years, several works targeting the development of pullulan and pullulan-based "active" coatings have demonstrated the suitability of this exopolysaccharide to act as an excellent vehicle for bioactive components and nutrients.[71] Indeed, pristine pullulan was claimed as an inherently antimicrobial biopolymer by Yuen.[11] Lately, this was also confirmed by Chlebowska-Śmigiel et al.,[72] who attributed the capability of pullulan to inhibit the growth of several microorganisms (molds, yeasts, and bacteria) to the high gas-barrier properties (O_2 and CO_2). In addition, Singh reported that pullulan improves the shelf life of the food as it cannot be readily assimilated as a carbon source by bacteria, molds and fungi responsible for spoilage of food.[20]

To improve the inherent antimicrobial attribute of pullulan coatings, several compounds of both synthetic and natural origin were used. For example, Gniewosz et al. incorporated thymol in pullulan coatings and the antimicrobial effect against different bacteria species (*Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella enteritidis*, and *Escherichia coli*) was evaluated on apples and mandarins.[73] The edible coating was able not only to prolong the shelf life of the tested fruits but also to improve their final appearance in terms of optical properties.

Trinetta et al. loaded pullulan coatings with a bacteriocin (Sakacin A) and tested its efficacy against epidemic clones of *Listeria monocytogenes* in turkey deli meat.[74] The authors demonstrated that sakacin A can migrate from the biopolymer into the food and inhibit the

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pathogen growth over 3 weeks of refrigerated storage. In another study, the same authors produced sakacin-based pullulan films blended with xanthan gums and locust bean.[75] These films exhibited good mechanical and optical properties, as well as antimicrobial effects, and were thus suggested as a valid alternative to oil-based plastic packaging materials.

Wu et al. prepared pullulan-based coatings in combination with antibrowning and antibacterial agents (glutathione and chitooligosaccharides, respectively).[48] The addition of the two active components effectively retarded enzymatic browning and inhibited microbial growth (i.e., psychrophilic bacteria) on apple slices (Fuji cultivar), thereby showing its potential to extend the shelf-life of apple slices and minimally processed fruits.

The antimicrobial activity of pullulan coatings on apples (Jonagored cultivar) was also evaluated by incorporating bioactive extracts from meadowsweet flower (*Filipendulae ulmariae flos*).[76] Pullulan active coatings effectively inhibited the growth of natural mesophilic microflora, the amount of which decreased by two logarithmic cycles at the end of storage. Moreover, the addition of the extracts to the coating significantly limited the growth of the *Rhizopus arrhizus* mold inoculated to the apples.

In recent years, the well-known antimicrobial properties of silver have stimulated researchers in the endeavor of creating active packaging materials based on biopolymers. Pinto and co-workers have developed thin films of pullulan loaded with Ag nanoparticles and tested their antifungal activity against *Aspergillus niger*.[77] The authors demonstrated that the antimicrobial pullulan/Ag films inhibited fungal growth. In addition, disruption of the spore cells of *A. niger* was probed for the first time, which was ascribed to the Ag nanoparticles dispersed as fillers in pullulan.

In addition to films and coatings, new materials with innovative and appealing performance have been conceived recently. Pullulan was used in combination with Ag to generate antimicrobial nanoparticles, where the biopolymer acted as both a stabilizing and reducing agent.[78] The authors successfully assessed the antibacterial and antibiofilm activities against food-borne and multidrug resistant pathogens such as *E. coli*, *L. monocyogenes*, *B. cereus*, *K. pneumonia*, and *P. aeruginosa*. Moreover, the antifungal activity was evaluated against fungal pathogens such as *Aspergillus spp.*, and *Penicillum spp*. In particular, the obtained results revealed that all bacterial and fungal pathogens were inhibited in a dose-dependent manner.

Electrospun hybrid nanofibers were prepared by blending pullulan and PVOH and incorporating silver nanoparticles as an antimicrobial agent.[79] The new material showed a good inhibitory effect against *S. aureus* and *E. coli*, showing its potential as a new preservative.

Electrospun nanofibers were also obtained by blending pullulan and β-cyclodextrin to encapsulate bioactive aroma compounds, namely perillaldehyde extracted from leaves of the plant *Perilla frutescens* with antimicrobial properties.[80] The authors developed a system able to retain the volatile compounds for months without losses if stored at ambient relative humidities and at different temperatures, as usually happens for food packaging materials. The active membranes were instead activated at high water activities ($a_w \geq 0.9$), being the surrounding humidity the trigger for the controlled release over time of the antimicrobial molecules.

Food-grade electrospun fibers from amaranth protein and pullulan blends were finally developed by Aceituno-Medina et al., who claimed these new materials as a potential option for the encapsulation of bioactives for functional food applications.[81]

An alternative route to produce antimicrobial pullulan films and coatings with potential application in food packaging has recently envisaged a chemical approach known as grafting.

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Fernandes et al., for example, used 3-aminopropyltrimethoxysilane as a metal alkoxide precursor to functionalize pullulan in correspondence of its hydroxyl groups.[82] Interestingly, the introduction of active aminopropyl groups into the pullulan polymeric backbone imparted antimicrobial activity against Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*). In a similar way, pullulan grafted with poly(3-acrylamidopropyl)trimethylammonium chloride was shown to inhibit the growth of *S. aureus*.[83]

Finally, Kanmani et al. investigated edible pullulan films blended with starch of different origin loaded with multiple probiotic bacterial strains.[84] Pullulan and pullulan/potato starch films were found to be the most suitable carrier matrices, with a maximum relative cell viability of 70–80% after 2 months of storage at 4 °C, confirming the potential use of pullulan and pullulan/starch films as effective delivery and carrier systems for probiotics.

EMERGING OPPORTUNITIES

Pullulan-based coatings on oil-based plastic films

Recent trends towards low-environmental-impact packaging materials have urged new solutions to tackle increasing issues such as the waste disposal and the increasing price of crude oil, together with the uncertainty related to crude oil's durability. Although many attempts have been made to replace oil-based polymers with bio-based materials, the gap in functional properties between these two classes (e.g., durability, mechanical, and water vapor barrier) is too wide to justify full turnover.

In our first work on this subject, we proposed the combination of plastics from petroleum and biopolymers through the deposition of a thin biopolymer coating on a plastic web as a possible early step in using biomacromolecules as food packaging materials.[85] According to the

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"packaging optimization" principle, the use of high-performance thin layers would allow down-gauging current packaging structures, e.g., laminates and/or co-extruded materials, without impairing (and, in fact, improving) the overall performance of the substrate beneath, at reasonable cost.[85] Several works embracing this approach have demonstrated its great potential to provide new, multifunctional structures with less environmental impact.[86–93] However, the potentiality of pullulan as a new biopolymer able to provide the oil-based plastic substrate of unprecedented functionalities has only been investigated in the last three years.

Wettability modifier coatings

In a first study, the wettability of pullulan and four other biopolymers (i.e., chitosan, gelatin, and two types of pectins) coated onto a poly(ethylene terephthalate) (PET) plastic substrate were investigated using a combined contact angle/image analysis (i.e., trigonometric-geometric) approach.[94] This allowed quantifying the contribution of each physicochemical phenomenon involved at the solid/liquid interface over a time span of 60 s. Absorption and spreading were identified as the major effects occurring upon water droplets deposition on the biopolymer coating surface. Arising from the observations made by the authors, pullulan turned out to be the most hydrophilic surface among the biopolymers tested, with the lowest contact angle values (30° and 23° immediately after droplet deposition and after 60 s, respectively) (Figure 3a and b). The surface energy analysis also revealed that pullulan behaved as a strong monopolar electron donor, similarly to synthetic polymers such as poly(ethylene oxide) (PEO) and PVOH, confirming the resemblance between pullulan and polymers derived from petroleum. It was also proved that the contact angle evolution over time largely accounted for spreading rather than absorption. This was first attributed to some of the aforementioned physicochemical features of

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this exopolysaccharide, in particular high hydroxyl content along the molecular skeleton (nine –OH groups per repeating unit—maltotriose) and lack of crystal domains, which concurrently made more favorable the interaction between the biopolymer and the water molecules. However, atomic force microscopy (AFM) allowed for the highlighting of another striking feature of pullulan, i.e., its very smooth surface (roughness ∼ 3 nm), with a presumed orientation of the molecular chains after the coating deposition (Figure 4a and b). Again, this observation was linked to two other distinct features of the pullulan molecule, viz its flexibility around the α-(1→6)-linkage and the absence of charged groups, both of which play a role in dictating the extent of folding and aggregation on the molecular level.

Findings arising from that study led to a more "market-oriented" research later on. The same research group indeed took advantage of previous observations to develop an innovative anti-fog coating specifically intended for food packaging applications.[95] In this sector, the formation of tiny water droplets on the surface of the package (especially fresh fruits and vegetables) is considered as detrimental for a suitable display of the product—the water droplets hide the contents of the package by scattering of the incident light in all directions. Dynamic contact angle measurements and specific anti-fog tests clearly demonstrated the superior anti-fog performance of the pullulan coating (coating thickness ∼ 0.5 μm) over uncoated poly(ethylene) (PE) and PE films loaded with commercially available anti-fog additives (Figure 5a–c). This was attributed to the capability of pullulan to form a continuous and even layer on the plastic substrate, while the commercial films had a here-and-there distribution. Unexpectedly, spectrophotometric analyses disclosed another peculiarity of pullulan coatings; the pullulan-coated samples exhibited transparency and haze values slightly larger and lower, respectively, than the neat PE. Such an improvement in the optical properties was also recognized by the

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naked eye. The authors attributed this effect to the smoothing of the original morphology of the polyolefin surface ensuing from the deposition of the pullulan coating. In turn, this yielded a reduction in the light scattering due to surface roughness effects. However, additional properties of the coating, such as the refractive index and inherent antireflectance, may have had a role, although this was not experimentally proved.

Oxygen barrier coatings

The target of high oxygen barrier packaging materials has long been pursued due to the need for new solutions and technologies allowing the control of oxygen-driven reactions (e.g., oxidation of fatty acids, degradation of vitamins) that may inevitably affect the quality attributes and shelf life of foods. Over the last decade, there has been a lot of development in the area of new barrier technologies in an attempt to provide more efficient solutions, e.g., compared with heavy and expensive materials such metal and glass containers.[96] Plastics have thereby risen as the preferential material to fulfill the requirements of high performance, low cost, and less environmental standing. Toward this goal, the deposition of very thin layers on the surface of oil-based materials has turned out to be one of the most promising avenues. For example, inorganic silicon-oxide thin coatings showed properties similar to glass, such as transparency, water-resistance, and very high barrier against gases. A more recent development concerned the so-called "hybrid coatings", i.e., thin layers obtained by the physical and or chemical coupling of an inorganic component (often given by a Si-based metal alkoxide) with an organic counterpart. Although several techniques may be adopted to generate these hybrids, the sol-gel technique has been recognized as the most feasible processing method for technically sound and commercially viable hybrid thin films and coatings.[97] So far, the organic phase has been mainly represented by

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an oil-derived polymer with high hydroxyl content on its backbone, such as PVOH and ethylene(vinyl alcohol) (EVOH), to boost the interaction of the inorganic and organic phases by covalent and/or hydrogen bonding at these sites.[98,99]

The use of biopolymers in place of oil-derived synthetic polymers to produce hybrid materials has been explored some years later.[100] Pullulan, in particular, was suggested as a suitable candidate for the generation of flexible, transparent, and high oxygen barrier nanostructured coatings for food packaging applications.[40] In a first attempt, pullulan was used in combination with a metal alkoxide with four ethyl groups susceptible of hydrolysis (i.e., tetraethoxysilane, TEOS), with the goal of achieving high oxygen barrier performance even at high relative humidity values. The authors demonstrated that the formation of the bionanostructured network occurred through self-assembly of the two phases driven by intermolecular hydrogen-bond formation at the interfaces of end-capped silanol groups generated from TEOS and of the pendant hydroxyl groups of the pullulan backbone (Figure 6). The resulting structure was thus viewed as a tightly packed, highly dense nano-sized three-dimensional hybrid network with the inorganic and the organic phases intimately mixed. The authors also pointed out that it is necessary to use the low amount of silica to avoid extensive cracking of the coatings, in order to maintain the protective effect toward the surrounding humidity. Final OTR values of the pullulan-based hybrid coatings were fully comparable to most common solutions available on the market, making pullulan, in association with silica, a viable alternative to the organic polymers derived from petrol for the development of oxygen barrier coatings, especially as intended for food packaging applications.

Pullulan was successively tested for its ability to generate bionanocomposite coatings.[101] In that work, natural montmorillonite (Na^+-MMT) was used as the nanosized filler for the

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improvement of the oxygen barrier properties in two distinct ways, i.e., (i) a more tortuous path for diffusion of the permeant and (ii) local changes in the polymer matrix properties (e.g., molecular mobility) at the interfacial (polymer−nanoparticle) regions. An inorganic/organic (I/O) ratio of ~ 0.4 was pinpointed as the best combination to achieve permeability values of the PET (12 μm)-coated films of 1.43 mL·μm·m^{-2}·(24h)$^{-1}$·atm^{-1} and 258 mL·μm·m^{-2}·(24h)$^{-1}$·atm^{-1} at 0% and 70% RH, respectively (coating thickness ~ 1 μm). The overall performance of the pullulan bionanocomposite was thus fully comparable with one of the most widely used oxygen barrier films, the PET/poly(vinylidene chloride) (PVDC) coating, which has an O$_2$TR ~ 7.5 mL·m^{-2}·(24 h)$^{-1}$ at 23 °C under dry conditions (PET thickness = 12 μm; coating thickness = 1.4 μm) Interestingly, the same coated films exhibited haze values of ~3 %, which is an adequate value for most application in the food packaging sector. In the same work by Introzzi et al.,[101] new condensed matter structures referred to as spherulites were observed for the first time on the pullulan coatings (in absence of filler) in forms of both feather-like formations and perfectly round particles (Figure 7a and b). As for the latter, they were thought of as high-order semicrystalline self-assemblies originated from thermodynamic incompatibility between pullulan and PET. In turn, this would have triggered a phase separation that leads to the aggregation of pullulan, which undergoes a partial crystallization with radial growth around a starting nucleus.

In a very recent investigation, the two aforementioned approaches (i.e., sol-gel and intercalation) were combined to produce self-assembled nanostructured biohybrid coatings with further enhanced oxygen barrier properties.[38] Results coming from XRD and TEM analyses suggested that a new supramolecular organization stemmed from the self-assembly between pullulan and the nanobuilding blocks Na$^+$−MMT. Most likely, pullulan molecules acquired a more ordered arrangement ensuing from the alignment of the biopolymer chains inside the clay

interlayers, with the nanosized "glass-like" silica network acting as reinforcement of the overall matrix (Figure 8a and b). Eventually this yielded outstanding oxygen barrier properties, which can be profitably exploited for many applications in the food packaging sector.

FUTURE CHALLENGES

Although it has many fascinating properties, pullulan has not been as adequately exploited as it deserves. Indeed, very few commercial products are currently on the market, with the largest share of the suggested applications being at laboratory or semi-industrial scale. The main reason for such commercial underdevelopment is its high price. Pullulan cost ranges between 25 and 30 US$/kg,[19,40] which is much higher than most biopolymers of both polysaccharides and protein origin. The high cost of pullulan is mainly linked to its production.

The industrial bioprocess for the production of pullulan involves the use of carbohydrates as carbon sources, because they allow high productivities and yields.[10,102] However, the selling prices for the commonly used substrates for production of most microbial polysaccharides, sugar and starch, are currently around 350 to 518 US$/ton and 279 to 310 US$/ton, respectively.[71] Since the substrate cost accounts for up to 40% of the total production costs of microbial polymers,[103] it appears obvious that a first strategy for reducing price is the use of inexpensive raw materials, such as wastes and byproducts. Although many efforts are currently being made in this direction,[1] some obstacles seem to make the path very arduous, such as the difficulty in guaranteeing their supply in terms of quantity and quality, the different nutrient composition of the raw materials, the presence of contaminants in the substrates, and accumulation of nonreacted components in the broth.[71]

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Another issue that contributes to the high price of pullulan is within the downstream processing of the fermentation broth, and is represented by the removal of the melanin pigment. Removal of melanin is usually performed using activated charcoal after which purification of pullulan can take place. The selection of melanin-free, high-yield strains could thereby represent, together with new alternative fermentation schemes, another valuable strategy to widen pullulan applications.[2]

To open new market opportunities, especially in the food packaging field, some technological aspects should be addressed in coming years. In particular, shortcomings linked to the high affinity of pullulan toward water molecules hinder several applications. Even though the hydrophilic nature of pullulan can be of advantage for several uses, it is also the reason for its high sensitivity to moist environments. Certainly, this would not allow its use in common packages if protective layers (e.g., polyolefin films) are placed on top of it, thus preventing inherent or enhanced properties (e.g., the oxygen barrier) from being compromised. Another aspect that may rule out pullulan from certain food packaging applications is its neutrality. The absence of any kind of charge on its backbone does not allow, for example, the establishment of ionic interactions with countercharged polymers and/or small molecules, which is the basis for the development of certain release systems, polyion-complex structures, and complex coacervates.[70,87]

Finally, another envisioned use of pullulan within the food packaging industry is as a natural sealing coating. Although the adhesive properties of pullulan have repeatedly been pointed out, there is no work dealing with this appealing application. In our team, research on this aspect is still ongoing. So far, we have observed the high tendency to seal of freshly-prepared-pullulan coatings on PET substrates. However this property is completely lost after two days of storage

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(unpublished data). Our observations prompted us to hypothesize that reorganization of pullulan chains occur over time, which would lead to the formation of crystalline domains and/or inaccessibility of hydroxyl groups, eventually making void the initial adhesion properties. This is in agreement with a recent study on the film-forming mechanism during the drying of aqueous pullulan solutions.[104]

Among several strategies (e.g., blending, nanotechnology), one valuable approach to impart new functionalities to pullulan derivatives (i.e., films and coatings) relies on derivatization.[1,9] Pullulan can be derivatized by grafting new chemical structures on its hydroxyl groups, thus providing unprecedented features. This kind of chemical modification has the advantage of providing tailored structures for each specific application. Several derivatization routes can be realized with pullulan, a summary of which has been reported in Table 2. With special reference to the food packaging field, the most relevant of these chemical modifications is carboxylation, which makes possible introducing negative charges on the pullulan backbone, and the isocyanate chemistry, which would allow new features such as thermoplasticity and resistance to water by the insertion of new urethane groups.[105]

CONCLUDING REMARKS

Packaging optimization and judicious use of plastics from fossil fuels are two pressing tasks for the tomorrow's research in food packaging. Biopolymers certainly represent a potential option to achieve this goal. Pullulan, in particular, offers important advantages over other polysaccharides and proteins due to its incomparable physicochemical properties. Unfortunately, its high price restricts its use to lab-scale or pilot-scale applications. Market penetration of already-existing technologies involving pullulan forcefully passes through two main tracks, namely improvement

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of the production process and enhancement of its multifunctionality. Therefore, innovative industrial applications can be reached if researchers at both the academic and industrial level are be able to find inspiration to creatively develop new routes and strategies enabling the generation of new high-performance pullulan materials with a higher level of sophistication. In such an endeavor, it is of paramount importance to aim at recyclable, environmental friendly, energy-saving, reliable, and cost-efficient solutions.

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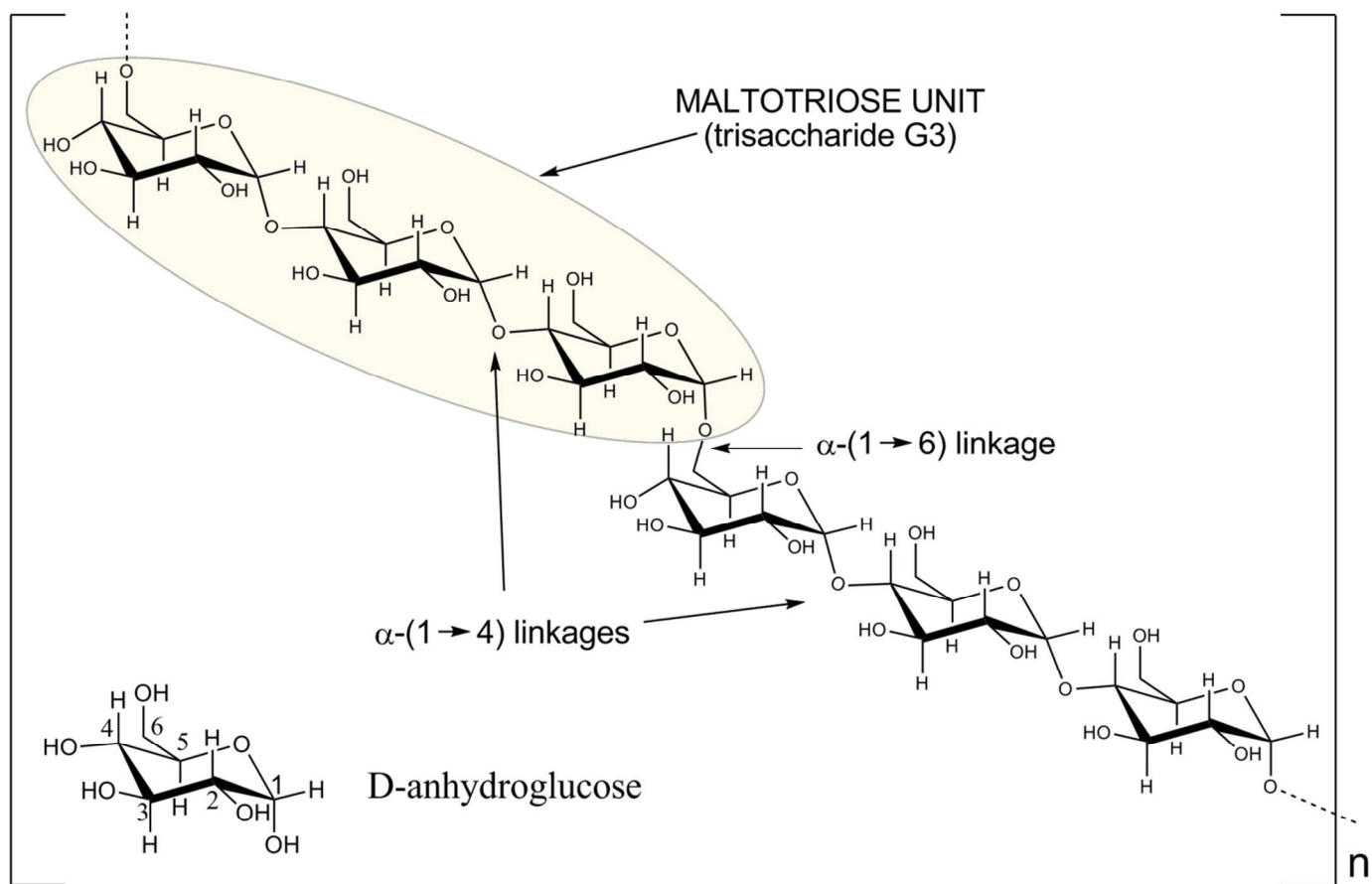
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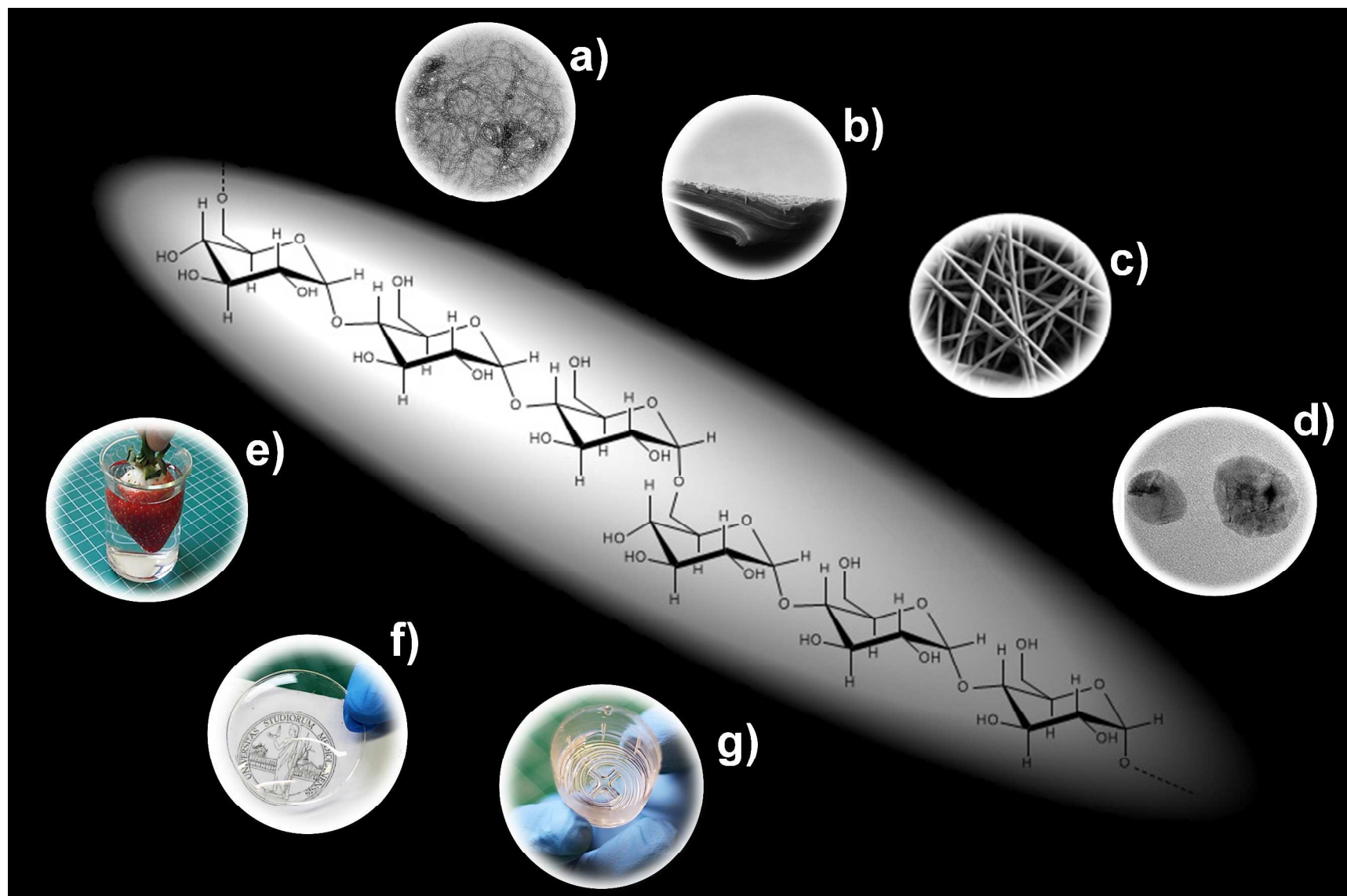46) Biliaderis, C. G.; Lazaridou, A.; Arvanitoyannis, I. *Carbohydr. Polym.* **1999**, *40*, 29–47.

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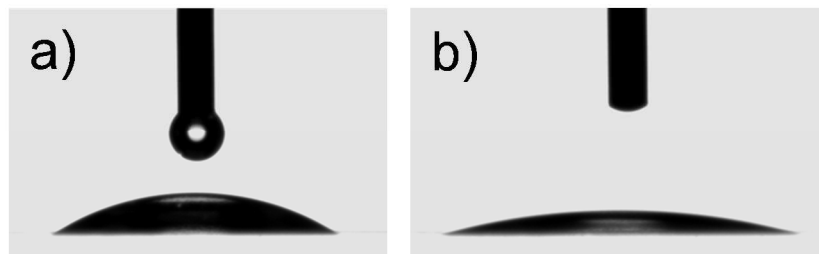
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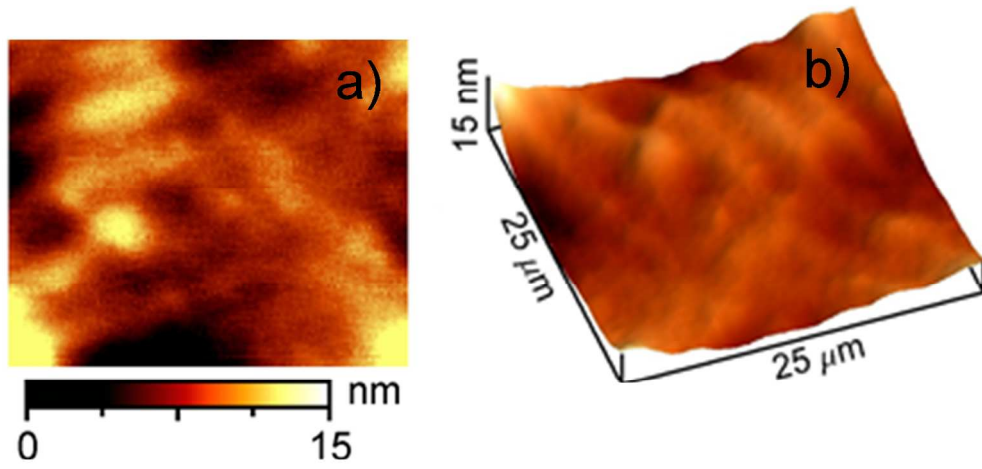93) Hult, E. L.; Iotti, M.; Lenes, M. *Cellulose* **2010**, *17*, 575–586.

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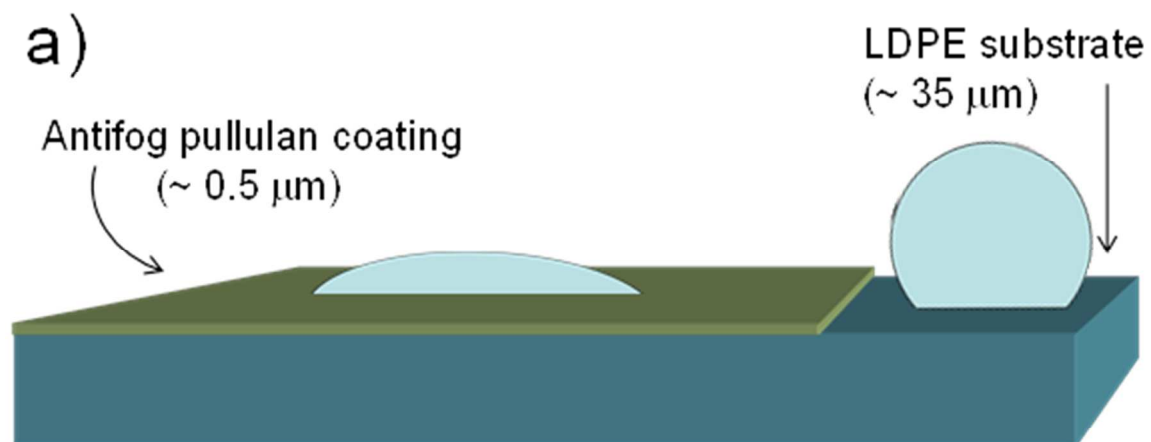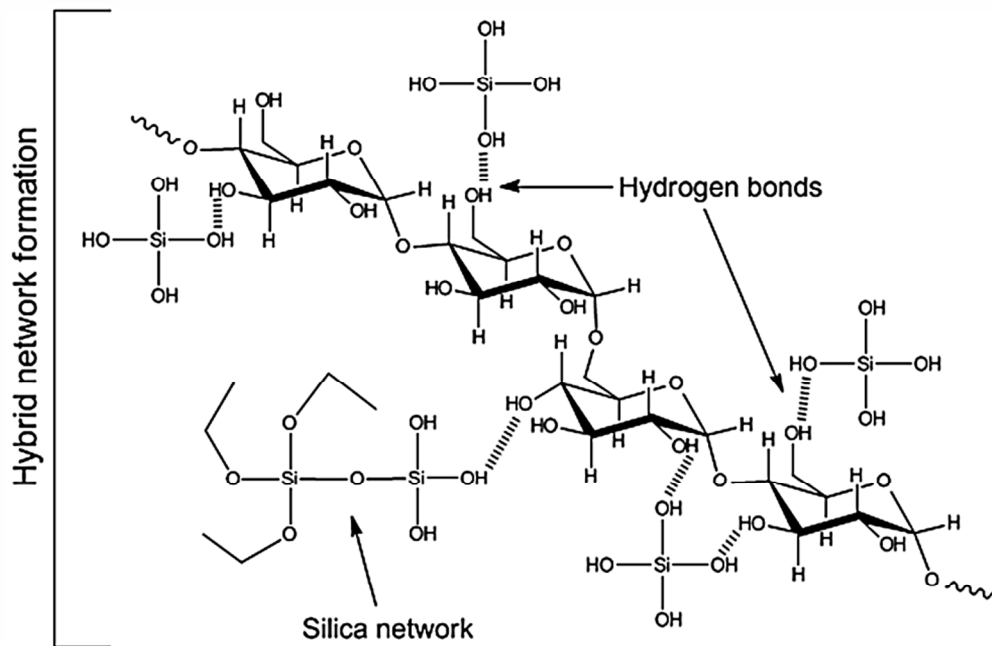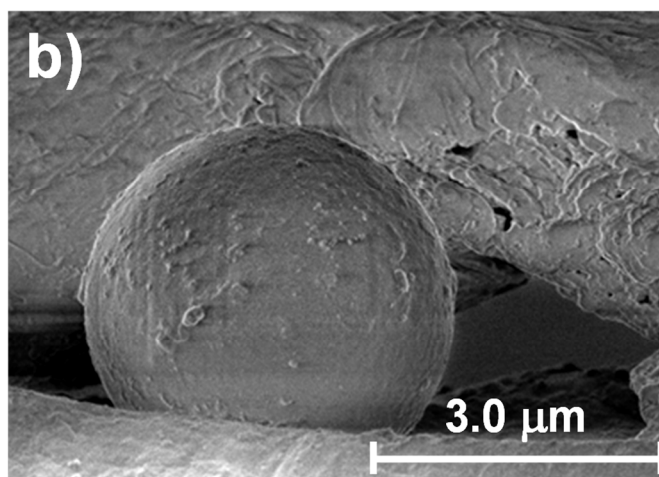105) Shibata, M.; Asahina, M.; Teramoto, N.; Yosomiya, R. *Polymer* **2001**, *42*, 59–64.

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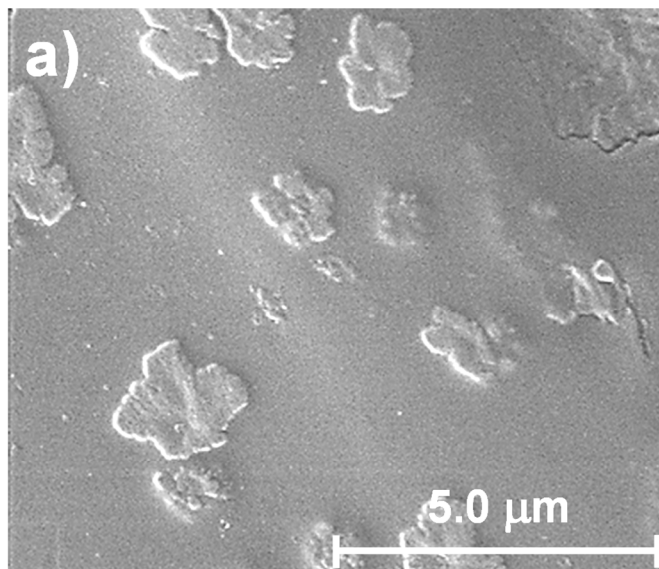(trisaccharide G3)

α-(1 ➔ 6) linkage

α-(1 ➔ 4) linkages

D-anhydroglucose

a)

b)

Na⁺-MMT platelets
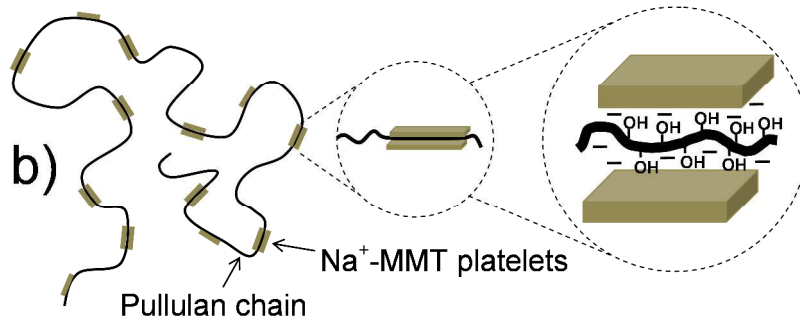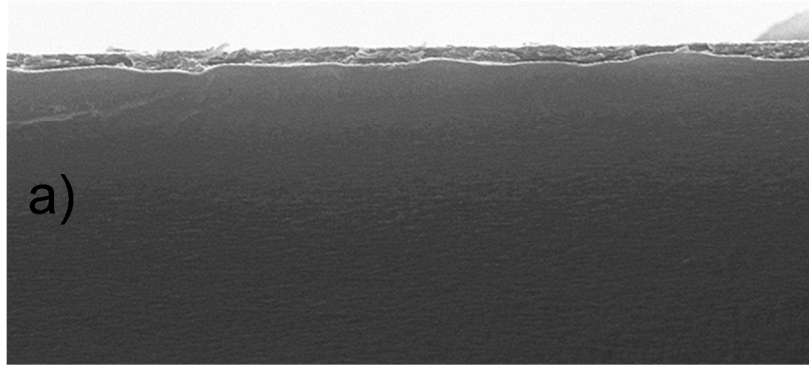
Pullulan chain

Film/coating material*	Investigated properties	Reference
Pullulan-soy protein	Adhesion to the food surface	Chlebowska-Śmigiel et al. [47]
Pullulan-caseinate	Thermal	Kristo et al. [50]
Pullulan-whey protein	Oxygen permeability, water vapor permeability, moisture content, water solubility, appearance	Gounga et al. [51]
Pullulan-hydroxypropyl methylcellulose	Thermo-mechanical	Prasad et al. [52]
Pullulan-whey protein	Thermo-mechanical	Gounga et al. [53]
Pullulan-alginate-carboxymethylcellulose	Water solubility	Tong et al. [54]
Pullulan-rice wax	Water vapor permeability, moisture sorption	Shih et al. [55]
Pullulan-sodium alginate	Thermo-mechanical	Xiao et al. [56]
Pullulan-sodium alginate	Rheological behavior	Xiao et al. [57]
Pullulan-gellan	Mechanical, water vapor permeability	Zhu et al. [58]
Pullulan-chitosan and pullulan-carboxymethyl chitosan	Mechanical, barrier (O_2 and H_2O), water solubility, color	Wu et al. [59]
Pullulan-sodium alginate	Moisture sorption, thermodynamic	Xiao et al. [60]
Pullulan-corn starch	Thermo-mechanical	Yan et al. [61]
Pullulan-gelatin	Mechanical, oxygen permeability	Zhang et al. [62]
Pullulan-carboxymethylgellan	Moisture sorption, mechanical, water vapor permeability	Zhu et al. [63]

Reaction	Type of derivatization on the –OH group of pullulan (P-OH)
Etherification	P-O–CH$_3$ (permethylation) P-O–(CH$_2$)$_{2-3}$–CH$_3$ (alkylation) P-O–CH$_2$–COOH (carboxymethylation) P-O–(CH$_2$)$_{2-3}$–CH$_2$–NH^{3+} (cationization) P-O–CH$_2$–CH$_2$–CN (cyanoethylation) P-O–(CH$_2$)$_{1-4}$–Cl (chloroalkylation) P-O–CH$_2$–CH$_2$–(S=O)–CH$_3$ (sulfinylethylation)
Isocyanate chemistry	P-O–CO–NH–CH$_2$–CH(OH) –CH$_3$ P-O–CO–NH–CH$_2$–CH$_2$–NH^{3+} P-O–CO–NH–R (R = phenyl or hexyl) P-O–CO–NH-phenyl
Oxidation	P-COOH (C$_6$ oxidation) Glycosidic ring opening (periodate oxidation)
Sulfation	P-O–SO$_3$Na
Esterification	P-O–CO–CH$_3$ (acetylation) P-O–CO–CH$_2$–Cl (chloroacetylation) P-O–CO–CH$_2$–CH$_2$–COOH (succinoylation) P-O–CO– (CH$_2$)$_{2-14}$–CH$_3$ (alkoylation)
Chlorination	P-CH$_2$–Cl (C$_6$ substitution)
Azido-pullulan	P-CH$_2$–N$_2$

Figure captions

Figure 1. Structure of pullulan and its repeating unit maltotriose. The basic monomer (D-anhydroglucose) is also depicted, with the position of each carbon atom indicated by a number.

Figure 2. Representative illustration of different forms of pullulan with potential application in the food packaging industry. Starting from its native organization as bundles (a) pullulan can be manipulated to generate thin layers on packaging materials such as plastics (b), electrospun nanofibers (c) (adapted from ref. 80), and micro- nano-particles doped with active compounds (d) (adapted from ref. 78); pullulan can also be used as an edible coating on food (e), to generate stand-alone films (f), and to fabricate three-dimensional objects (g).

Figure 3. Water contact angle of a pullulan coating laid on a PET substrate immediately after droplet deposition (a) and after 60 s (b) (adapted from ref. 94).

Figure 4. AFM height (a) and three-dimensional (b) images ($25 \times 25 \ \mu m^2$) of a pullulan-biocoated PET surface (adapted from ref. 94).

Figure 5. Schematic representation of the change in wettability promoted by a pullulan coating compared to the uncoated substrate (low-density polyethylene, LDPE) (a). Expanded polystyrene (EPS) trays with an uncoated (b) and pullulan-coated (c) LDPE top after removal from the refrigerator (7 days at ~ 4 °C) into the laboratory environment (~ 20 °C). The foggy appearance of the uncoated sample is clearly visible.

Figure 6. Schematic representation of the hybrid network formation between pullulan and tetraethoxysilane (TEOS) by a typical sol-gel reaction.

Figure 7. SEM micrographs of spherulitic formations on the pure pullulan coating surfaces: feather-like dendritic crystals at 10k× magnification (a) and perfectly spherical self-associations at 15k× magnification (b). Adapted from ref. 101.

Figure 8. Scanning electron microscopy (SEM) of the pullulan/Na$^+$-MMT nanostructured biohybrid coating on a PET substrate (a). Hypothesized supramolecular organization generated by the self-assembly of pullulan chains and the inorganic filler. Adapted from ref. 38.

Table captions

Table 1. Examples of pullulan-based edible films and coatings and their functional properties with potential application in food packaging.

FOOTNOTE: *The presence of plasticizers (e.g., glycerol, sorbitol) is here omitted.

Table 2. Summary of most relevant substitution reactions on pullulan backbone.

FOOTNOTE: Adapted from ref. 13