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Review

# Perspectives for biocatalysts in food packaging

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Since the market for minimally processed foods is broadening, packaging is being implemented in strategies that actively contribute to the food preservation and transformation concepts. One opportunity is given by the incorporation of biocatalysts, usually in prefabricated carriers, where they can fully retain their activity. These biocatalysts can be repeatedly reused within the packaging or, under controlled conditions, migrate to the product. The potential use of packages composed of classic polymers with functional surfaces or highly swellable biopolymers is being successfully tested, showing good perspectives for the controlled release when directly used in contact with the product or as part of combined strategies. Moreover, new nanostructured materials (modified clays, LBL assemblies, electrospun fibres) are being implemented as part of more innovative solutions. This review summarizes the most recent developments and upcoming possibilities to incorporate biocatalysts in polymers traditionally used in food packaging applications and in some recently introduced biopolymers.

## Introduction

Enzymes or biocatalysts are molecules that make possible specific chemical reactions, without being consumed during the process. The use of these molecules with high added-value is of key importance in biotechnological processes, so they are extensively used by the food industry (Olempska-Beer, Merker, Ditto, & DiNovi, 2006; Panesar, Panesar, Singh, Kennedy, & Kumar, 2006). In some industrial applications, the direct use of biocatalysts can have disadvantages due to their sensitivity to processing conditions and to trace levels of substances that can actuate as inhibitors, resulting in short operational life or inactivation. Their application confined in different tailored carriers usually provides the enzymes with additional stability under strong working conditions (pH, temperature) and an adequate environment for the repeated use or the controlled release of the biocatalysts (Kandimalla, Tripathi, & Ju, 2006). In addition, though enzymes generally only play a technological role, not being present in the final product, the European Directive 95/2/EC already classifies some enzymes, like invertase (E1103) and lysozyme (E1105), as food additives that might be used and labelled as food ingredients.

In contrast with other applications of immobilized enzymes, for their correct incorporation into food packages, more stress should be placed in the need for controlling migration rates of the bioactive molecules, being reusability less important. The enzyme load and release, should allow the attainment of the desired effect without compromising the quality and safety of the final product.

This review highlights the known applications of biocatalysts in food packaging and, furthermore, reports some of the newest technologies described in the literature to functionalize polymers and/or produce nanostructured materials with potential applications for the development of enzyme-based active food packaging solutions. From Table 1, where the most recent developments in this research field have been summarized, it can be inferred that the main interest arises from approaches related with food safety. However, a lot of attention is also being paid to the potential advantages that can be obtained carrying out transformations in prepacked foods, specially if the transformation has positive effects over the quality of the food (active role) or the health of the consumer (bioactive role).

## Polymers as support for active and bioactive substances

Since minimal processing is being implemented for a broad range of foodstuffs, food preservation requires the use of integral solutions where the packaging is playing

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**Table 1. Overview of the reference list describing applications of biocatalysts included in materials typically used in food packaging systems, as sorted by methodology and objectives of the implemented strategy**

| Methods | Biocatalyst | Polymer | Packaging | Objectives | References |
|---|---|---|---|---|---|
| Covalent bonding | Lactase | LDPE | X | Food transformation | Goddard *et al.*, 2007 |
| Covalent bonding | Glucose oxidase | PP | X | Antimicrobial | Vartiainen *et al.*, 2005 |
| Adsorption | Invertase<br>Glucoamylase | PVOH | | Food transformation | Rebros *et al.*, 2006<br>Rebros *et al.*, 2007 |
| Adsorption | Lysozyme | Chitosan | | | Hoven *et al.*, 2007 |
| Adsorption in montmorillonite | Invertase<br>Alpha-amylase<br>Glucoamylase | | | Food transformation | Gopinath & Sugunan, 2007 |
| Entrapment | Naringinase | PVOH | X | Food transformation | Del Nobile *et al.*, 2003 |
| Entrapment | Lysozyme | PVOH<br>Nylon<br>Cellulose | X | Antimicrobial | Appendini & Hotchkiss, 1997 |
| Entrapment | Lysozyme | PVOH | X | Antimicrobial | Conte *et al.*, 2006<br>Conte *et al.*, 2007 |
| Entrapment in multilayers | Lysozyme | PVOH | X | Controlled release (antimicrobial) | Buonocore, Del Nobile, Panizza, Corbo, *et al.*, 2003<br>Buonocore, Del Nobile, Panizza, Bove, *et al.*, 2003<br>Buonocore *et al.*, 2004<br>Buonocore *et al.*, 2005 |
| Entrapment in laminates | Glucose oxidase<br>Catalase | LDPE | X | Oxygen scavenging | Andersson *et al.*, 2002 |
| Entrapment | Lysozyme | Zein<br>Soy protein | X | Antimicrobial | Padgett *et al.*, 1998 |
| Entrapment | Lysozyme | Zein | X | Antimicrobial | Mecitoglu *et al.*, 2006 |
| Entrapment | Lysozyme | Zein | X | Antimicrobial | Gucbilmez *et al.*, 2007 |
| Entrapment | Lysozyme | Chitosan | X | Antimicrobial | Park *et al.*, 2004 |
| Entrapment | Lysozyme | Whey proteins | X | Antimicrobial | Min *et al.*, 2005 |
| Entrapment | Lysozyme | Na-alginate | X | Antimicrobial | Cha *et al.*, 2002 |
| Entrapment | Lysozyme | Gelatin | X | Antimicrobial | Bower *et al.*, 2006 |
| Entrapment | Lactase | Gelatin | | Biosensor | Sungur & Akbulut, 1994<br>Logoglu *et al.*, 2006 |
| LBL assemblies | Lysozyme | | X | Antimicrobial | Rudra *et al.*, 2006 |
| Deposition by MAPLE | Lysozyme | | | Antimicrobial | Purice *et al.*, 2007 |
| Electrospinning | Glucose oxidase | PVOH | | Biosensor | Ren *et al.*, 2006 |

a more active role. In this context, active packaging strategies are being developed aiming at the integration and/or controlled release of active molecules in conventional or renewable packaging systems, giving them an added-value.

Evidences in the literature, widely support the incorporation of antimicrobial food components and additives into packaging materials to reinforce the hurdle concept in the food production chain. These compounds have been proven to have an antimicrobial or bacteriostatic effect on spoilage of foods by pathogenic microorganisms, yeasts and fungi. Across these works, several strategies intended to incorporate low molecular weight antimicrobial food components and bacteriocines to different films used for food packaging applications are described (Almenar, Del Valle, Catala, & Gavara, 2007; Lopez, Sanchez, Batlle, & Nerin, 2007; Mauriello, De Luca, La Storia, Villani, & Ercolini, 2005; Rodríguez, Batlle, & Nerín, 2007; Suppakul, Miltz, Sonneveld, & Bigger, 2003). In addition, the diffusion of different antimicrobial compounds from packaging materials has been extensively reviewed by Han (2000). Up to now, some silver-based and triclosan-based antimicrobial additives have already been introduced in commercialized films in Japan and the United States. Those films have applications in paper and plastic containers intented for food technology. However, the existence of many regulatory constrains seems to limit the expansion of this technology, especially in Europe, where the Directive on active packaging technologies is currently under preparation.

Another group of compounds that is also receiving a great deal of attention in the development of active packages is that of the molecules with ability to act as oxygen or radical scavengers. The contact of small amounts of oxygen with sensitive foodstuffs might produce their oxidation, the development of rancidity, colour changes and off-flavours. The incorporation of oxygen or radical scavengers (Nerin *et al.*, 2006) in the packaging represents an active packaging solution able to eliminate residual oxygen in the headspace and/or prevent the contact of food with atmospheric oxygen entering the package. Commercial oxygen scavengers are usually highly oxidizable substances, as iron powders, incorporated to the packaging inside individual

sachets (Lopez-Rubio *et al*., 2004). Oxygen scavengers offer advantages for the packaging of lots of different products and new approaches are being successfully commercialized, included in the label or directly in the food contact side of the packaging (*Packaging gets active: additives lead the way, 2004*). One company (Bioka Ltd.) is already commercializing a biocatalyst-based technology making use of glucose oxidase in an individual sachet, under the brand name Bioka, offered as an oxygen scavenging technology intended for the preservation of food quality.

Finally, the incorporation in the packaging of certain enzymes as cholesterol reductase or lactase can increase the value of a foodstuff during storage, and also makes it match the necessities of target consumers with health problems. However, in spite of the great industrial interest, active or bioactive packages where transformations take place by the action of some fixed or released enzymes have not been implemented to date.

On the other hand, the immobilization of biomolecules on traditional polymers coming from non-renewable sources has been limited by practical constrains. Their structure is commonly inert, and consequently, if they are going to act as carriers of enzymes, their surface must be activated before. However, natural biopolymers (Cutter, 2006), which have a structure similar to the molecules traditionally used as solid supports for immobilized substances, are starting to replace the traditional matrices and have good perspectives to be included in conventional processing lines. Some biopolymers, particularly proteins and polysaccharides, are a good barrier against hydrophobic compounds like most lipids, oxygen and some flavours. Additionally, bio-based polymers are considered sustainable resources, since they are renewable, and most of them biodegrade relatively easy and many are also edible. Limitations for the extended use of these biopolymers for food packaging applications are their relatively poor mechanical and water vapor barrier properties, difficulties for their lamination, and their hydrophilicity, which makes them sensitive to humidity and readily soluble in polar matrixes. In these materials, the sorption of water can also produce strong plasticization, which among other detrimental effects for the packaging can induce the uncontrolled release of the immobilized active substances. However, this uncontrolled release can be avoided by chemical linking of the active substance to the polymer, as described later in this review work.

Some biopolymers that can be used as edible films and coatings are zein, gluten, milk proteins, gelatin, starch, pectinates and cellulose–ethers. Chitosan presents good perspectives to be used as edible film or coating, though its use blended with conventional polymers has also been described (Lee, Park, & Lee, 2004). Two additional advantages of chitosan are its biocide effect and film forming properties, however, its approval as food contact material in Europe is still pending. Edible coatings cannot be typically considered packages, but physical food protecting barriers, that can additionally act as carriers of active and/or bioactive substances. Actually, the inclusion of oxygen scavengers or antimicrobial enzymes in those films is considered a tool of key interest in order to extend product shelf-life or to induce controlled transformations in the foodstuffs. Therefore, the incorporation of biocatalysts can make more attractive and active applications for edible polymers in the near future.

## Chemical bonding

The covalent binding of biocatalysts to a solid support is one of the most extended methods of enzyme immobilization, and one of the most commonly applied in bioprocesses. The growing needs of the industry are expanding the number of technologies developed for the covalent bonding of active components, as has been recently reviewed by Goddard and Hotchkiss (2007). As the surface of most food contact polymers used at the moment is inert, their functionalization becomes necessary in most cases. Side-groups of the enzyme molecules not involved with the active centre and capable of holding a covalent union if they are exposed to the appropriate surface, i.e. non-hydrophobic aminoacids like lysine, cysteine, tyrosine and histidine, are good candidates to take part in the covalent bonding. During the binding, the active site must be protected, which is usually done by fixing a molecule of the substrate or a competitive inhibitor.

The covalent union between the polymer and the biocatalyst is based on the chemical activation of a surface and the attachment of nucleophilic groups of the proteins. This union transforms the polymer in a stable carrier that, in the best case, does not leach the protein to the surroundings. This is particularly important in food contact applications, since a full immobilization could avoid the arrival of undesirable or unintended compounds to the food matrix.

Polymer films covalently bonded to active or bioactive compounds would provide with excellent solutions for in-package food processing, however, their application in food technology is still limited by the high toxicity of the chemicals required to activate the surface of the polymers. So is the case of the system proposed by Goddard, Talbert, and Hotchkiss (2007) for the immobilization of beta-D-galactosidase by covalent attachment to activate low density polyethylene (LDPE). In this system, with excellent enzyme activity retention, the bonds between the lactase and the functionalized LDPE withstood heat also in the presence of ionic denaturants, showing high stability and low probability of enzymatic leakage into the food. However, compounds like sodium cyanoborohydride and glutaraldehyde were used to activate the LDPE surface. Glucose oxidase, known to have antimicrobial activity and widely used in biosensors, has also been covalently immobilized onto amino- and carboxyl-plasma-activated bioriented polypropylene films displaying high activity yields, since they remarkably inhibited the growth of *Bacillus subtilis* and completely inhibited the growth of *Escherichia coli* (Vartiainen, Ratto, & Paulussen, 2005). The last approach was

performed using carbodiimide and glutaraldehyde chemistries to activate the polymer surface. However, both examples reveal the very good perspectives of these active polymers in food packaging applications.

Weaker unions than the covalent bonds are those provided by weak secondary forces such as hydrogen bonds, ionic or hydrophobic interactions, or the Van der Waals forces. These unions are obtained when the enzymes are adsorbed onto solid supports. Advantages of this technology are the usual retention of optimal bioconversion rates, and the possibility to modulate the exchange between the protein and the matrix by changing the pH. In addition, enhanced enzyme stabilities are commonly reported when they are immobilized by adsorption methods. Indeed, the reversibility of binding has been perceived as a valuable advantage in processes where enzymes had to be purified and isolated from complex mixtures, though it is of less interest in food packaging applications. Numerous ion exchangers have been developed for different purposes. Among them, silica supports with superficial chemical modifications are probably the solids most extensively used for enzyme adsorption. Nevertheless, and to the best of our knowledge, food packaging applications of this type of materials have not been described yet.

Further approaches deal with ionic modifications of biopolymers, such as chitosan. Hoven, Tangpasuthadol, Angkitpaiboon, Vallapa, and Kiatkamjornwong (2007) introduced charges directly in chitosan surfaces *via* methylation and reductive alkylation. The surface, negatively charged with *N*-sulfofurfuryl groups, exhibited selective protein adsorption against negatively and positively charged proteins, as ribonuclease and lysozyme. Additionally, ionic modifications of polyvinyl alcohol (PVOH), might induce adsorption of enzymes into non-toxic hydrogels, as it is the case reported for LentiKats® (Jekel, Buhr, Willke, & Vorlop, 1998) providing with low diffusion rates for biocatalysts if they were additionally cross-linked. This matrix has been successfully used to immobilize enzymes typically used in food transformations, like glucoamylase and invertase (Rebros, Rosenberg, Mlichova, & Kristofikova, 2007; Rebros, Rosenberg, Mlichova, Kristofikova, & Paluch, 2006), but the application of these active hydrogels in food packaging has not been reported yet.

More innovative methodologies attempt to immobilize enzymes onto clays through adsorption or grafting, finding acceptable activities of enzymes important for food transformation processes (Gopinath & Sugunan, 2007) as alpha-amylase, glucoamylase and invertase, when included in batch or bed reactors. Layered inorganic compounds, such as clays, are typically used as micro and nanofillers in polymers, reinforcing the tensile strength, modulus, barrier and heat distortion of selected matrixes, as packaging films (Abreu, Losada, Angulo, & Cruz, 2007; Lagaron, Cava, Cabedo, Gavara, & Gimenez, 2005). Furthermore, the low resistance to humidity and low barrier properties of the biopolymers might be widely overcome with the addition of nanofillers, since exfoliated nanoclays increase the tortuosity factor in films, increasing their barrier properties. Consequently, since clays are reported to act efficiently as carriers for enzymes, new approaches might be expected in the next future dealing with the adsorption of biocatalysts into clays incorporated to biopolymers, trying to achieve the controlled release of molecules of interest in food technology and food packaging (Rhim & Ng, 2007).

## Physical retention methods

The entrapment of active substances in polymers represents a simple and flexible tool for the physical retention of the molecules. Macromolecules, as biocatalysts and cells might be entrapped in porous solid matrices as gelatin, chitosan, agar, alginate, carragenate, starch, and others (Bodalo *et al*., 1991; Gombotz & Wee, 1998). Most of them are natural biodegradable polymers widely used in food technology and food packaging.

Indeed, hydrogels (Durso & Fortier, 1996; Navratil *et al*., 2002; Panesar *et al*., 2006), with a high water content, provide adequate environments for enzyme activity. Enzymes are usually entrapped between the polymer monomers. Polymerization is induced by the incorporation of chemical substances (i.e. bivalent cations to polymerize alginates), temperature shifts, or by exposure to light. To establish a network between the enzyme molecules and the polymers, cross-linkers, as glyoxal, glutaraldeyde, formaldehyde or transglutaminase, are frequently used. The entrapment occurs in a gel or between synthetic fibres, allowing the biocatalyst to interact with the environment. Diffusion restrictions difficulting the interaction of the enzymes with large substrates might be overcome with the entrapment inside more porous polymers, like acetate cellulose fibres (Jawaheer, White, Rughooputh, & Cullen, 2003).

Generally, full enzyme activity might be retained since negligible changes in the protein structure are induced during the entrapping process. Enzymes typically used in food transformations already have been successfully entrapped in beads or membranes. As an example, calcium alginate beads have been used for the entrapment of alpha-amylase (Ertan, Yagar, & Balkan, 2007) or beta-galactosidase (Haider & Husain, 2007) but up to now there are no commercial applications related to food packaging of these systems. In some cases, increased stability towards pH or heat has even been reported for some entrapped biocatalysts. This is indeed a remarkable feature, since the leaching of biomolecules into the food matrix and/or cross-reactions with other food components, such as lipids or proteins could, among other things, reduce the effectiveness of the entrapped biomolecules, a certain stabilization through immobilization would be particularly recommended in food systems. That is the case of the tyrosinase, whose entrapment in alginate or gelatin has been stated to produce a higher thermal stability. A broad pH activity profile of

tyrosinase was also described after its entrapment in alginate and polyacrylamide gels (Munjal & Sawhney, 2002). Furthermore, beta-glucosidase from *Pyrococcus furiosus* has been entrapped in gelatin with a remarkable increase in thermostability and a better yield of transglucosylation than the native enzyme (Nagatomo, Matsushita, Sugamoto, & Matsui, 2005). Moreover, in the references Logoglu, Sungur, and Yildiz (2006), Sungur and Akbulut (1994) and other from the same authors, several solutions for the cross-linking of beta-galactosidase in gelatin with glutaraldehyde and/or chromium(III) acetate have been proposed, obtaining remarkable improvements in the activity of the enzyme when it was cross-linked with the chromium(III) acetate.

Following those principles, Appendini and Hotchkiss (1997) first developed antimicrobial films with lysozyme retained in different polymers, as polyvinyl alcohol (PVOH), nylon pallets and cellulose triacetate films. Lysozyme has also been immobilized in PVOH films using glyoxal and glutaraldehyde as cross-linkers (Conte, Buonocore, Bevilacqua, Sinigaglia, & Del Nobile, 2006; Conte, Buonocore, Sinigaglia, & Del Nobile, 2007). Those authors provided formulations with antimicrobial properties against *Micrococcus lysodeikticus*, ascribing the antimicrobial activities to the enzyme. Through adequate enzyme/glutaraldehyde ratios, satisfactory immobilization yields were described and inappreciable leakage of the enzymes has been detected. PVOH matrices cross-linked with glutaraldehyde have also been effectively used to entrap naringinase (Del Nobile *et al*., 2003), with high enzyme activity loads. The authors not only outlined the potential use of the active films to improve the sensory properties of grape fruit juices but also remarked the importance of considering limitations due to the film constitution, as the enzyme was only able to hydrolyze the naringin diffusing into the film. Indeed, this is the main constrain when trying to develop bioactive films to be applied in food transformation processes.

Among all the revised literature, mainly the works of Buonocore, Conte, Corbo, Sinigaglia, and Del Nobile (2005), Buonocore *et al*. (2003), Buonocore, Del Nobile, Panizza, Corbo, and Nicolais (2003), and Buonocore *et al*. (2004) are clearly focused on strategies to control the release of antimicrobial enzyme molecules from highly swellable polymers. These authors tried to control the release of antimicrobial compounds to the food surface by the use of multilayer films composed by a control layer in contact with the food, an active matrix layer containing the active substance, and a barrier layer to prevent the release of the active substance to the external environment. While, once swollen, the matrix layer would have a very fast and almost uncontrolled diffusion, the control layer would control the flux of release of the active substance. This should be done adjusting the thickness and diffusivity rates of the material, and taking into account the characteristics of microbial spoilage of the food products. These works reported the suitability of highly swellable polymers, being able to modulate the release kinetics of lysozyme and nisin by changing the degree of the chemically induced cross-linking. These active films were also able to inhibit the growth of *M. lysodeikticus*, *Alicyclobacillus acidoterrestris*, and *Saccharomyces cerevisiae*. In a more recent work, Buonocore *et al*. (2005) compared multilayer structures using PVOH films as matrix layer with PVOH monolayers, reporting a considerable reduction in the release rate of lysozyme in the former structure and also showing that a satisfactory control of enzyme release might be achieved after cross-linking with glyoxal.

In an original attempt to produce films with the capacity to absorb oxygen, Lehtonen, Karilainen, and Aaltonen (1991) proposed an invention where they included oxidoreductases, catalase and glucose oxidase within laminate layers. This material was claimed to be effective to prolong the food shelf-life and showed oxygen scavenging activity. Furthermore, cellulose-based laminates with glucose oxidase and catalase, together with glucose and $CaCO_3$ have been included between aluminium and low density polyethylene layers following an industrial process at Tetra Pak (Lund, Sweden) (Andersson, Andersson, Adlercreutz, Nielsen, & Hornsten, 2002), similar to that described for obtaining the conventional Tetra Brik laminates. The enzymes under dry conditions showed extreme resistance to high temperatures during processing (up to 325 °C for several short cycles), since the scavenging activity was almost fully preserved. Therefore, good perspectives to tolerate the industrial lamination processes could be presumed and those materials showed very good perspectives to be implemented in-packaging lines.

Entrapped enzymes could also have an application in edible coatings (Cagri, Ustunol, & Ryser, 2004). Former works by Padgett, Han, and Dawson (1998) studied the inhibitory effect of lysozyme, nisin, and EDTA after their incorporation in heat-press or cast corn zein films, or in soy protein isolates. All the films were effective against *Lactobacillus plantarum*, exhibiting larger inhibition zones in the films obtained by solution casting than the films obtained applying high temperatures and pressure. Furthermore, the addition of EDTA increased the antimicrobial activity against Gram-negative bacteria, like *E. coli*. Mecitoglu *et al*. (2006) also reported the activity of zein films where partially purified lysozyme from hen egg had been incorporated. Although lysozyme was partially aggregated in the zein films, the release rate at 4 °C varied between 7 and 29 U/cm$^2$/min, increasing at high lysozyme concentrations. These films showed antimicrobial activity against *B. subtilis* and *L. plantarum* and became effective against *E. coli* when EDTA was added. Gucbilmez, Yemenicioglu, and Arslanoglu (2007) also incorporated partially purified lysozyme into zein films, obtaining excellent results when chickpea albumin extract and EDTA were added to the active film. Lysozyme and chickpea albumin extract also increased the inherent free radical scavenging activity of the zein films. The chickpea albumin extract also reduced

the releasing rate of lysozyme (between 1.5- and 3.5-fold) and significantly improved distribution of the enzyme in the film. The films containing the three active components (lysozyme, EDTA and chickpea albumin extract) were effective against *E. coli* and *B. subtilis*.

Park, Daeschel, and Zhao, 2004 reported the incorporation of lysozyme to chitosan films, in a development aimed at improving the inherent antimicrobial properties of chitosan. The films, prepared by solvent evaporation, showed enhanced antimicrobial activity against *E. coli* and *Streptococcus faecalis*, while their water vapor permeability was not affected by lysozyme incorporation. On the other hand, the tensile strength and percent elongation decreased. Indeed, a certain electrostatic interaction between chitosan amino groups and protein anionic groups has been postulated, and used for the entrapment of soluble proteins in aqueous food processing streams (Wibowo, Velazquez, Savant, & Torres, 2005). This property allows the retention of high amounts of proteins in the chitosan matrix and reveals this polymer as an exceptional holder for enzymes of interest also in food packaging.

Edible films and coatings formed by whey protein isolates containing lysozyme were also tested against *Listeria monocytogenes*, total aerobes, yeast and molds, showing very good antimicrobial properties on culture media and on cold-smoked salmon during 35 days storage at 4 and 10 °C (Min, Harris, Han, & Krochta, 2005). Differences between the effectivity against *L. monocytogenes* of films and coatings were observed, but both types of films were effective at 4 and 10 °C. The incorporation of lysozyme also increased the plasticity and oxygen permeability of the films.

Cha, Choi, Chinnan, and Park (2002) prepared Na-alginate films containing nisin, lysozyme, EDTA and grape fruit seed extracts with a broad spectra of activity. They inhibited Gram-positive and Gram-negative bacteria. In that work, Na-alginate-based films exhibited larger inhibitory zones than κ-carrageenan-based films. Also films with grape fruit seed extracts were effective against the indicator microorganisms.

Furthermore, Bower, Avena-Bustillos, Olsen, McHugh, and Bechtel (2006) produced fish-skin gelatin containing hen egg-white lysozyme that provided satisfactory antimicrobial activity against Gram-positive bacteria. However, other properties like the gel clarity and strength were impacted at the concentrations required to avoid the growth of bacteria. Such approaches indeed offer exceptional possibilities to improve the handling of edible coatings by increasing the stability of perishable polymers, and/or to extend the shelf-life of foods.

## Nanoscaled immobilization systems

Regarding its application to enzyme immobilization systems, nanostructures provide new perspectives to enhance the available surface contact area and modify the mass transfer, which probably are the most important factors affecting the effectiveness of these systems. *N*anoscaled immobilization systems would strongly modify the performance of these materials. The high surface area to volume ratios, the Brownian motion of nanoparticles, the possibility of producing porous structures, the self-assembling properties of some nanostructures and the possibility of producing optimised materials compatible with typical polymers used in food packaging or edible films or coatings provide opportunities that are generating high expectations in many research areas, including food packaging.

Up to now, numerous tailored nanoscaled materials have been developed as support for biomolecules (Kim, Grate, & Wang, 2006). Surface modified silica nanoparticles, gold and carbon nanotubes have already been employed through different approaches (Merkoci, 2006; Qhobosheane, Santra, Zhang, & Tan, 2001). Actually, the techniques reported for enzyme immobilization in nanostructured materials, are based on the traditional techniques reported above. However, nanoparticles in the form of powders present a number of problems, and they are not applied in food packaging strategies.

Similar approaches refer to organic ultrathin films, like self-assembled mono-layers (SAM), Langmuir–Blodgett (LB) films, and layer-by-layer (LBL) assemblies, which are already widely used for the immobilization of biomaterials (Ariga, Nakanishi, & Michinobu, 2006). Multilayer coatings of layers with a thickness in the nanoscale range can be made by sequential adsorption of oppositely charged polyelectrolytes on a solid support. A polypeptide multilayer nanofilm obtained by this procedure and constituted by poly(L-glutamic acid) (negatively charged at acidic pH) and hen egg-white lysozyme (net positive charge at acidic pH), was evaluated for its antimicrobial properties. The film was reported to inhibit the growth of *Micrococcus luteus* in liquid medium and showed good perspectives in strategies involved in food preservation, due to the easy application of the layer-by-layer assemblies (Rudra, Dave, & Haynie, 2006). Additionally, a simple control of the releasing rate of the enzyme by adjusting the amount of film layers was demonstrated. Matrix assisted pulsed laser evaporation (MAPLE) is another technique that can also provide deposition of enzyme layers with thicknesses in the nanometer range, but their application is limited to electronics, although films with efficient catalytic activity of, for example, lysozyme have been recently reported and could be applied for food packaging (Purice, Schou, Kingshott, Pryds, & Dinescu, 2007).

Another novel technology to produce polymeric ultrafine fibres is by means of electrospinning. This technology generates fibers, which are easier to handle and reduce the health concerns that arise from nanoscaled powders. This technique consists of generation of a strong electric field around a droplet of a polymer, or a polymer blend, at the tip of a syringe as this flows through. The droplet is deformed and forms a jet, that is accelerated in the direction of the counter electrode where it lands in the shape of a fibre-based mat structure (Fong, Chun, & Reneker, 1999).

Structures generated by electrospinning can be nanobeads, nanofibres or microfibres (Torres-Giner, Gimenez, & Lagaron, in press). These structures allow the entrapment of bioactive molecules yielding high activities, as demonstrated for the particular case of alpha-chymotrypsin (Jia *et al*., 2002) and with glucose oxidase (Ren *et al*., 2006), which is particularly useful for biosensor applications. A great number of matrices can be electrospun, including biopolymers, which result in an array of opportunities open for the biofunctionalization of food packaging materials expected to be developed in the near future. Some authors have already started to explore the possibilities of this technique, with special good perspectives for tailored drug delivery systems, so for example, Zeng *et al*. (2005) have reported the retardation of the release of the enzyme luciferase by coating electrospun fibres with other polymers. Coaxial electrospinning might therefore provide new opportunities for reinforcing several properties of polymers and biopolymers.

## Future trends

Packaging technologies are being implemented to play an important role in food preservation strategies. Contrary to chemical compounds, enzymes present the advantage of being highly specific catalysts not consumed during processing. The use of antimicrobial enzymes and enzymes with oxygen scavenging properties in active food packaging further develops the hurdle concept in food processing. Moreover, certain bioconversions might be conveniently implemented in prepacked foodstuffs to transform foods for special purposes during storage. Nowadays classic polymers and new hydrogels with functionalized surfaces, together with highly swellable biopolymers are being tested as holders for biocatalysts, showing good perspectives for the controlled release in food packaging when directly used in contact with the product or as part of multilayers. Furthermore, the way traditional polymers or biopolymers are functionalized and used in food processing is changing, as novel nanostructured materials (modified clays, electrospun fibres) are being implemented in innovative solutions. Taking into consideration the already existing approaches and the performances of new biopolymers that will be surely approved soon in Europe for food contact applications, we expect this research field to further expand, achieving commercial applications in parallel with the developments in edible films and coatings.

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## References

Abreu, D. A. P., Losada, P. P., Angulo, I., & Cruz, J. M. (2007). Development of new polyolefin films with nanoclays for application in food packaging. *European Polymer Journal, 43*(6), 2229–2243.

Almenar, E., Del Valle, V., Catala, R., & Gavara, R. (2007). Active package for wild strawberry fruit (*Fragaria vesca* L.). *Journal of Agricultural and Food Chemistry, 55*(6), 2240–2245.

Andersson, M., Andersson, T., Adlercreutz, P., Nielsen, T., & Hornsten, E. G. (2002). Toward an enzyme-based oxygen scavenging laminate. Influence of industrial lamination conditions on the performance of glucose oxidase. *Biotechnology and Bioengineering, 79*(1), 37–42.

Appendini, P., & Hotchkiss, J. H. (1997). Immobilisation of lysozyme on food contact polymers as potential antimicrobial films. *Packaging Technology and Science, 10*, 271–279.

Ariga, K., Nakanishi, T., & Michinobu, T. (2006). Immobilization of biomaterials to nano-assembled films (self-assembled monolayers, Langmuir–Blodgett films, and layer-by-layer assemblies) and their related functions. *Journal of Nanoscience and Nanotechnology, 6*(8), 2278–2301.

Bodalo, A., Gomez, E., Gomez, J. L., Bastida, J., Maximo, J. F., & Diaz, F. (1991). Comparison of different methods of beta-galactosidase immobilization. *Process Biochemistry, 26*(6), 349–353.

Bower, C. K., Avena-Bustillos, R. J., Olsen, C. W., McHugh, T. H., & Bechtel, P. J. (2006). Characterization of fish-skin gelatin gels and films containing the antimicrobial enzyme lysozyme. *Journal of Food Science, 71*(5), 141–145.

Buonocore, G. G., Conte, A., Corbo, M. R., Sinigaglia, M., & Del Nobile, M. A. (2005). Mono- and multilayer active films containing lysozyme as antimicrobial agent. *Innovative Food Science & Emerging Technologies, 6*(4), 459–464.

Buonocore, G. G., Del Nobile, M. A., Panizza, A., Bove, S., Battaglia, G., & Nicolais, L. (2003). Modeling the lysozyme release kinetics from antimicrobial films intended for food packaging applications. *Journal of Food Science, 68*(4), 1365–1370.

Buonocore, G. G., Del Nobile, M. A., Panizza, A., Corbo, M. R., & Nicolais, L. (2003). A general approach to describe the antimicrobial agent release from highly swellable films intended for food packaging applications. *Journal of Controlled Release, 90*(1), 97–107.

Buonocore, G. G., Sinigaglia, M., Corbo, M. R., Bevilacqua, A., La Notte, E., & Del Nobile, M. A. (2004). Controlled release of antimicrobial compounds from highly swellable polymers. *Journal of Food Protection, 67*(6), 1190–1194.

Cagri, A., Ustunol, Z., & Ryser, E. T. (2004). Antimicrobial edible films and coatings. *Journal of Food Protection, 67*(4), 833–848.

Cha, D. S., Choi, J. H., Chinnan, M. S., & Park, H. J. (2002). Antimicrobial films based on Na-alginate and kappa-carrageenan. *Food Science & Technology, 35*(8), 715–719.

Conte, A., Buonocore, G. G., Bevilacqua, A., Sinigaglia, M., & Del Nobile, M. A. (2006). Immobilization of lysozyme on polyvinylalcohol films for active packaging applications. *Journal of Food Protection, 69*(4), 866–870.

Conte, A., Buonocore, G. G., Sinigaglia, M., & Del Nobile, M. A. (2007). Development of immobilized lysozyme based active film. *Journal of Food Engineering, 78*(3), 741–745.

Cutter, C. N. (2006). Opportunities for bio-based packaging technologies to improve the quality and safety of fresh and further processed muscle foods. *Meat Science, 74*(1), 131–142.

Del Nobile, M. A., Piergiovanni, L., Buonocore, G. G., Fava, P., Puglisi, M. L., & Nicolais, L. (2003). Naringinase immobilization in polymeric films intended for food packaging applications. *Journal of Food Science, 68*(6), 2046–2049.

Durso, E. M., & Fortier, G. (1996). Albumin-poly(ethylene glycol) hydrogel as matrix for enzyme immobilization: biochemical characterization of crosslinked acid phosphatase. *Enzyme and Microbial Technology, 18*(7), 482–488.

Ertan, F., Yagar, H., & Balkan, B. (2007). Optimization of alpha-amylase immobilization in calcium alginate beads. *Preparative Biochemistry & Biotechnology, 37*(3), 195–204.

Fong, H., Chun, I., & Reneker, D. H. (1999). Beaded nanofibres formed during electrospinning. *Polymer, 40*(16), 4585–4592.

Goddard, J. M., & Hotchkiss, J. H. (2007). Polymer surface modification for the attachment of bioactive compounds. *Progress in Polymer Science*, *32*(7), 698–725.

Goddard, J. M., Talbert, J. N., & Hotchkiss, J. H. (2007). Covalent attachment of lactase to low-density polyethylene films. *Journal of Food Science*, *72*(1), 36–41.

Gombotz, W. R., & Wee, S. F. (1998). Protein release from alginate matrices. *Advanced Drug Delivery Reviews*, *31*(3), 267–285.

Gopinath, S., & Sugunan, S. (2007). Enzymes immobilized on montmorillonite K 10: effect of adsorption and grafting on the surface properties and the enzyme activity. *Applied Clay Science*, *35*(1–2), 67–75.

Gucbilmez, C. M., Yemenicioglu, A., & Arslanoglu, A. (2007). Antimicrobial and antioxidant activity of edible zein films incorporated with lysozyme, albumin proteins and disodium EDTA. *Food Research International*, *40*(1), 80–91.

Haider, T., & Husain, Q. (2007). Calcium alginate entrapped preparations of *Aspergillus oryzae* beta galactosidase: its stability and applications in the hydrolysis of lactose. *International Journal of Biological Macromolecules*, *41*(1), 72–80.

Han, J. H. (2000). Antimicrobial food packaging. *Food Technology*, *54*(3), 56–65.

Hoven, V. P., Tangpasuthadol, V., Angkitpaiboon, Y., Vallapa, N., & Kiatkamjornwong, S. (2007). Surface-charged chitosan: preparation and protein adsorption. *Carbohydrate Polymers*, *68*(1), 44–53.

Jawaheer, S., White, S. F., Rughooputh, S. D. D. V., & Cullen, D. C. (2003). Development of a common biosensor format for an enzyme based biosensor array to monitor fruit quality. *Biosensors and Bioelectronics*, *18*(12), 1429–1437.

Jekel, M., Buhr, A., Willke, T., & Vorlop, K. D. (1998). Immobilization of biocatalysts in LentiKats. *Chemical Engineering & Technology*, *21*(3), 275.

Jia, H. F., Zhu, G. Y., Vugrinovichm, B., Kataphinan, W., Reneker, D. H., & Wang, P. (2002). Enzyme-carrying polymeric nanofibres prepared via electrospinning for use as unique biocatalysts. *Biotechnology Progress*, *18*(5), 1027–1032.

Kandimalla, V. B., Tripathi, V. S., & Ju, H. X. (2006). Immobilization of biomolecules in sol–gels: biological and analytical applications. *Critical Reviews in Analytical Chemistry*, *36*(2), 73–106.

Kim, J., Grate, J. W., & Wang, P. (2006). Nanostructures for enzyme stabilization. *Chemical Engineering Science*, *61*(3), 1017–1026.

Lagaron, J. M., Cava, D., Cabedo, L., Gavara, R., & Gimenez, E. (2005). Increasing packaged food quality and safety: (II) nanocomposites. *Food Additives and Contaminants*, *22*(10), 994–998.

Lee, C. H., Park, H. J., & Lee, D. S. (2004). Influence of antimicrobial packaging on kinetics of spoilage microbial growth in milk and orange juice. *Journal of Food Engineering*, *65*(4), 527–531.

Lehtonen, P., Karilainen, U., & Aaltonen, P. (1991). Glucose oxidase food treatment and storage method. US Pt. No. 4996062.

Logoglu, E., Sungur, S., & Yildiz, Y. (2006). Development of lactose biosensor based on beta-galactosidase and glucose oxidase immobilized into gelatin. *Journal of Macromolecular Science – Pure and Applied Chemistry*, *43*(3), 525–533.

Lopez, P., Sanchez, C., Batlle, R., & Nerin, C. (2007). Development of flexible antimicrobial films using essential oils as active agents. *Journal of Agricultural and Food Chemistry*, *55*(21), 8814–8824.

Lopez-Rubio, A., Almenar, E., Hernandez-Munoz, P., Lagaron, J. M., Catala, R., & Gavara, R. (2004). Overview of active polymer-based packaging technologies for food applications. *Food Reviews International*, *20*(4), 357–387.

Mauriello, G., De Luca, E., La Storia, A., Villani, F., & Ercolini, D. (2005). Antimicrobial activity of a nisin-activated plastic film for food packaging. *Letters in Applied Microbiology*, *41*(6), 464–469.

Mecitoglu, C., Yemenicioglu, A., Arslanoglu, A., Elmaci, Z. S., Korel, F., & Cetin, A. E. (2006). Incorporation of partially purified hen egg white lysozyme into zein films for antimicrobial food packaging. *Food Research International*, *39*(1), 12–21.

Merkoci, A. (2006). Carbon nanotubes in analytical sciences. *Microchimica Acta*, *152*(3–4), 157–174.

Min, S., Harris, L. J., Han, J. H., & Krochta, J. M. (2005). *Listeria monocytogenes* inhibition by whey protein films and coatings incorporating lysozyme. *Journal of Food Protection*, *68*(11), 2317–2325.

Munjal, N., & Sawhney, S. K. (2002). Stability and properties of mushroom tyrosinase entrapped in alginate, polyacrylamide and gelatin gels. *Enzyme and Microbial Technology*, *30*(5), 613–619.

Nagatomo, H., Matsushita, Y., Sugamoto, K., & Matsui, T. (2005). Preparation and properties of gelatin-immobilized beta-glucosidase from *Pyrococcus furiosus*. *Bioscience, Biotechnology and Biochemistry*, *69*(1), 128–136.

Navratil, M., Gemeiner, P., Klein, J., Sturdik, E., Malovikova, A., Nahalka, J., et al. (2002). Properties of hydrogel materials used for entrapment of microbial cells in production of fermented beverages. *Artificial Cell Blood Substitutes and Immobilization Biotechnologies*, *30*(3), 199–218.

Nerin, C., Tovar, L., Djenane, D., Camo, J., Salafranca, J., Beltran, J. A., et al. (2006). Studies on the stabilization of beef meat by a new active packaging containing natural antioxidants. *Journal of Agricultural and Food Chemistry*, *54*, 7840–7846.

Olempska-Beer, Z. S., Merker, R. I., Ditto, M. D., & DiNovi, M. J. (2006). Food-processing enzymes from recombinant microorganisms – a review. *Regulatory Toxicology and Pharmacology*, *45*(2), 144–158.

Packaging gets active: additives lead the way. *Plastics Additives & Compounding*March/April, 2004, 22–25.

Padgett, T., Han, I. Y., & Dawson, P. L. (1998). Incorporation of food-grade antimicrobial compounds into biodegradable packaging films. *Journal of Food Protection*, *61*(10), 1330–1335.

Panesar, P. S., Panesar, R., Singh, R. S., Kennedy, J. F., & Kumar, H. (2006). Microbial production, immobilization and applications of beta-D-galactosidase. *Journal of Chemical Technology and Biotechnology*, *81*(4), 530–543.

Park, S. I., Daeschel, M. A., & Zhao, Y. (2004). Functional properties of antimicrobial lysozyme–chitosan composite films. *Journal of Food Science*, *69*(8), 215–221.

Purice, A., Schou, J., Kingshott, P., Pryds, N., & Dinescu, M. (2007). Characterization of lysozyme films produced by matrix assisted pulsed laser evaporation (MAPLE). *Applied Surface Science*, *253*(15), 6451–6455.

Qhobosheane, M., Santra, S., Zhang, P., & Tan, W. H. (2001). Biochemically functionalized silica nanoparticles. *Analyst*, *126*(8), 1274–1278.

Rebros, M., Rosenberg, M., Mlichova, Z., & Kristofikova, L. (2007). Hydrolysis of sucrose by invertase entrapped in polyvinyl alcohol hydrogel capsules. *Food Chemistry*, *102*(3), 784–787.

Rebros, M., Rosenberg, M., Mlichova, Z., Kristofikova, L., & Paluch, L. (2006). A simple entrapment of glucoamylase into LentiKats((R)) as an efficient catalyst for maltodextrin hydrolysis. *Enzyme and Microbial Technology*, *39*(4), 800–804.

Ren, G. L., Xu, X. H., Liu, Q., Cheng, J., Yuan, X. Y., Wu, L. L., et al. (2006). Electrospun poly(vinyl alcohol)/glucose oxidase biocomposite membranes for biosensor applications. *Reactive & Functional Polymers*, *66*(12), 1559–1564.

Rhim, J. W., & Ng, P. K. W. (2007). Natural biopolymer-based nanocomposite films for packaging applications. *Critical Reviews in Food Science & Nutrition*, *47*(4), 411–433.

Rodríguez, A., Batlle, R., & Nerín, C. (2007). The use of natural essential oils as antimicrobial solutions in paper packaging. Part II. *Progress on Organic Coating*, *60*, 33–38.

Rudra, J. S., Dave, K., & Haynie, D. T. (2006). Antimicrobial polypeptide multilayer nanocoatings. *Journal of Biomaterials Science – Polymer Edition*, *17*(11), 1301–1315.

Sungur, S., & Akbulut, U. (1994). Immobilization of beta-galactosidase onto gelatin by glutaraldehyde and chromium (III) acetate. *Journal of Chemical Technology and Biotechnology*, *59*(3), 303–306.

Suppakul, P., Miltz, J., Sonneveld, K., & Bigger, S. W. (2003). Antimicrobial properties of basil and its possible application in food packaging. *Journal of Agricultural and Food Chemistry*, *51*(11), 3197–3207.

Torres-Giner, S., Gimenez, E., & Lagaron, J. M. Characterization of the morphology and thermal properties of zein, prolamine nanostructures obtained by electrospinning. *Food Hydrocolloids*, in press.

Vartiainen, J., Ratto, M., & Paulussen, S. (2005). Antimicrobial activity of glucose oxidase-immobilized plasma-activated polypropylene films. *Packaging Technology and Science*, *18*(5), 243–251.

Wibowo, S., Velazquez, G., Savant, V., & Torres, J. A. (2005). Surimi wash water treatment for protein recovery: effect of chitosan-alginate complex concentration and treatment time on protein adsorption. *Bioresource Technology*, *96*(6), 665–671.

Zeng, J., Aigner, A., Czubayko, F., Kissel, T., Wendorff, J. H., & Greiner, A. (2005). Poly(vinyl alcohol) nanofibres by electrospinning as a protein delivery system and the retardation of enzyme release by additional polymer coatings. *Biomacromolecules*, *6*(3), 1484–1488.