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Review

Chitosan application for active bio-based films production and potential in the food industry: Review

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ABSTRACT

During the past decade, there was an increasing interest to develop and use bio-based active films which are characterized by antimicrobial and antifungal activities in order to improve food preservation and to reduce the use of chemical preservatives. Biologically active bio-molecules such as chitosan and its derivatives have a significant potential in the food industry in view of contaminations associated with food products and the increasing concerns in relation with the negative environmental impact of conventional packaging materials such as plastics. Chitosan offers real potential for applications in the food industry due to its particular physico-chemical properties, short time biodegradability, biocompatibility with human tissues, antimicrobial an antifungal activities, and non-toxicity. Thus, chitosan-based films have attracted serious attention in food preservation and packaging technology. This is mainly due to a fact that chitosan exhibits high antimicrobial activity against pathogenic and spoilage micro-organisms, including fungi, and both Gram-positive and Gram-negative bacteria. The aim of the present review was to summarize the most important information on chitosan from its bioactivity point of view and to highlight various preparative methods used for chitosan-based active bio-films and their potential for applications in the food preservation and packaging technology.

1. Introduction

The modern food industry is facing challenges and requires specific approaches to surmount theme. One of these challenges is related to the packaging of food products with a short shelf life period. Although the use of conventional packaging materials such as plastics and their derivatives is effective for food preservation, they create serious environmental problems that continue to present the food industry as a source of pollution and social concerns. This problematic requires that all stakeholders in this industry and especially scientists specializing in the food engineering and packaging to seek alternatives to overcome this serious problem which is related to the packaging materials. A non-negligible aspect which is the total cost of the final product is also related to the packaging materials because it is well known that the contribution of the packaging to the product total cost is highly significant. So, the search for more economical packaging materials is a very important subject in the food industry.

Edible bio-based films have been investigated for their abilities to avoid moisture loss or water absorption by the food matrix, oxygen penetration to the food material, aromas loss and solute transports (Dutta, Tripathi, Mehrotra, & Dutta, 2009). Until now, the edible and biodegradable bio-based films are always not meant to totally replace the conventional packaging materials (Schou et al., 2005). However, the use of active bio-based films as packaging materials is still one of the most promising ways for effective methods of maintaining food quality. One of the most perspective active bio-film is the one based on chitosan combined with different materials such as plant and animal proteins, polysaccharides and antimicrobial peptides (bacteriocin) such as nisin and divergicin which is a new class bacteriocin produced by *Carnobacterium divergens* (Tahiri et al., 2004; Tahiri, Desbiens, Lacroix, Kheadr, & Fliss, 2009).

Chitosan; a linear polysaccharide consisting of (1,4)-linked 2-amino-deoxy-β-d-glucan, is a deacetylated derivative of chitin, which is the second most abundant polysaccharide found in nature after cellulose. Chitosan has been found to be non-toxic, biodegradable, biofunctional, biocompatible and was reported by several researchers to have strong antimicrobial and antifungal activities (Darmadji & Izumimoto, 1994; Jo, Lee, Lee, & Byun, 2001). Chitosan has been compared with other biomolecule-based active films used as packaging materials and the reported results showed that chitosan has more advantages because of its antibacterial activity and bivalent minerals chelating ability (Chen, Zheng, Wang, Lee, & Park,

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2002). Chitosan films have been successfully used as a packaging material for the quality preservation of a variety of foods (Ouattara, Simard, Piette, Begin, & Holley, 2000). Antimicrobial films have been prepared by including various organic acids and essential oils in a chitosan matrix, and the ability of these bio-based films to inhibit the growth of indigenous (lactic acid bacteria and *Enterobacteriaceae*) or inoculated bacteria (*Lactobacillus sakei* and *Serratia liquefaciens*) onto the surfaces of vacuum-packed cured meat products have been investigated. Release of organic acids (acetic and propionic acid) was found to be initially fast, when the gradient of ion concentration between the inside of the polymer matrix and the outside environment was high, then decreased as the release of acids progressed. At the same time, it was shown that the antimicrobial activity of the bio-based films under study did not affect growth and activity of lactic acid bacteria, whereas the growth of *Enterobacteriaceae* and *S. liquefaciens* was delayed or completely inhibited after storage during 21 days at 4 °C. Strongest inhibition was observed on surfaces with lower water activity values (bologna) onto which acid release was slower, and with films containing cinnamaldeyde, as a result of its greater antimicrobial activity under these conditions (Quintavalla & Vicini, 2002). Recently, a chitosan–starch film has been prepared using microwave treatment which may find potential application in the food packaging technology (Dutta et al., 2009).

The aim of the present review was to highlight the potential of chitosan as ingredient for active bio-based films production and to summarize the different methods used for chitosan-based films preparation and their perspectives in the modern food packaging technology.

2. Antimicrobial activity

Several studies showed that chitosan is characterized by an antimicrobial activity against a wide range of target microorganisms. Chitosan antimicrobial activity varies considerably with the type of chitosan; particularly the degree of deacetylation, molecular weight, the target organism and the conditions of the medium in which it is applied; especially pH, ionic strength and presence of solutes susceptible to react with chitosan through electrostatic interaction and/or covalent binding which can screen or completely block the reactivity of the active amine group. However, even if the available literature information on chitosan antimicrobial activity vary somewhat and, occasionally, contradictory findings have been reported, it is generally recognized that yeasts and moulds are the most sensitive group to chitosan, followed by Gram-positive and Gram-negative bacteria. A study on the effect of chitosan on yeast growth was reported and authors demonstrated that baker's yeast *Saccharomyces cerevisiae* showed halted fermentation activity by chitosan concentration as little as 3.6 mg/L in a buffer system solution (Ralston, Tracey, & Wrench, 1964). Also, similar antifungal activity has been reported against the mould *Fusarium solani*, the growth of which was stopped by 4 mg/L chitosan in a liquid nutrient medium (Kendra & Hadwiger, 1984). Variation in sensitivity between closely related microorganisms was illustrated in an experiment in which phytopathogenic fungi were screened for sensitivity to chitosan in liquid media. *Cytosporina sp*. isolate was completely inhibited by 75 mg/L chitosan, while a second isolate of the same genus was completely unaffected by chitosan concentration up to 1000 mg/L (Allan & Hadwiger, 1979). All these experimental findings are highly dependent of several intrinsic and extrinsic factors that affect the antimicrobial and antifungal activity of chitosan. It has been shown that low molecular weight chitosan with an average molecular weight less than 10 kDa have greater antimicrobial activity than native chitosan of high molecular weight (Uchida, Izume, & Ohtakara, 1989). This behaviour can be explained by a fact that low molecular weight chitosan is more soluble in aqueous media than high molecular weight chitosan and this solubility is of great importance to react with the active sites of the targeted microorganisms. However, even if low molecular weight chitosan is suitable, nevertheless a degree of polymerisation of at least seven basic units (glucosamine) is required; lower molecular weight fractions have little or no antibacterial or antifungal activity. Highly deacetylated chitosans are more antimicrobial than those with a higher proportion of acetylated amino groups. High deacetylation degree increases chitosan solubility and charge density. These two factors are important for chitosan adhesion to the bacterial cell. Low pH values (up to 5.5) increase the antimicrobial activity of chitosan because of its higher solubility and protonation in the acidic pH interval. A synergetic effect of chitosan positive charge and acidic media (low pH) has been also pointed out by several researchers. Temperature effect on chitosan antimicrobial activity was also reported. It was shown that at 37 °C the antimicrobial activity of chitosan was significantly higher than at refrigeration temperatures (Tsai & Su, 1999). This can be explained by the temperature effect on chitosan solution viscosity which decreases as temperature increases. However, it is well recognized that the surrounding matrix has the greatest influence on the antimicrobial activity of chitosan. Because of its unique cationic character, chitosan has the potential to bind to many different food components such as alginates, pectins, proteins, inorganic polyelectrolytes such as polyphosphate, and positively charged ion species through donor/acceptor interaction. In complex food matrices, the above mentioned particularity can decrease chitosan antimicrobial effect (Kubota & Kikuchi, 1998). This factor is a good indication that the promising results obtained *in vitro* with model systems in buffers or microbiological media are not necessarily applicable in real food systems which are complex matrices.

3. Factors influencing antibacterial activity of chitosan films

3.1. Effect of pH

It has been shown that the antimicrobial activity of chitosan and chitosan-based films increases by decreasing pH. This effect can be considered as synergetic for the reasons of the hurdle effect of the acid stress on the bacterial cells (Rhoades & Roller, 2000). Chitosan is a unique cationic polysaccharide in the nature, it has the particularity to bind to bacterial cell wall through electrostatic interaction and may cause damage by perturbing solute or nutrients transport to the cell (Liu, Du, Wang, & Sun, 2004). The mechanism of the antimicrobial activity of chitosan and its derivatives is well studied. As theory, it has been suggested that the positive charge of the amine group (NH_3^+) at pH values lower than the pKa (pH $<$ 6.3) at which this functional group carries 50% of its total electric charge allows the interactions with negatively charged microbial cell membranes; a phenomenon which is susceptible to cause a leakage of intracellular constituents (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001). However, even if this is a well recognized explanation of chitosan antimicrobial effect, there is no direct evidence demonstrating this behaviour against bacteria.

3.2. Intrinsic factors

Chitosan intrinsic factors significantly affect its antimicrobial and antifungal activity. It has been demonstrated that lower molecular weight chitosan with an average molecular weight less than 10 kDa have greater antimicrobial activity than native chitosan (Dutta et al., 2009) and that the degree of polymerisation of at