Effect of Glucose Oxidase Incorporation in Chitosan Edible Films Properties

Lopes, M. I.[1] , Martins, J. T.[2] , Fonseca, L. P.[1], Vicente, A. A.[2] *

[1]Centre for Biological and Chemical Engineering, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal.

[2]IBB – Institute of Biotechnology and Biotechnology, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.

*E-mail: avicente@deb.uminho.pt, Phone: +351.253.604419, Fax: +351.253.678986

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Abstract. *Demand for biodegradable and environmentally friendly packaging has draw attention into the development of biodegradable, polymer based edible packaging films. Edible films' functionality can be enhanced by incorporating active substances such as biocatalysts. The main objective of this work was to evaluate the effect of GOx incorporation in the mechanical and barrier properties of chitosan edible films.*

Films were prepared by the casting method using 2% (w/v) chitosan with 25% (w/w) glycerol, containing 1.1% (v/v) of a glucose oxidase solution (0.02% (w/v)). Water vapor, oxygen and carbon dioxide permeabilities were evaluated and Fourier Transform InfraRed (FTIR) analysis was performed.

The results of the mechanical tests showed that tensile strength and elongation-at-break values of chitosan films increased with the incorporation of glucose oxidase (from 9.6 to 10.6 MPa and from 65.5 to 71.6 %, respectively), however no significant ($p<0.05$) changes were observed. From permeability tests it was observed that the barrier properties of chitosan films were not affected by the enzyme incorporation. FTIR analysis showed a specific chemical interaction between functional groups of glucose oxidase and bioactive groups of chitosan.

These results suggest that chitosan films with incorporated GOx could be used as active packaging as a strategy for extension of food's shelf-life.

Keywords. Edible film, chitosan, glucose oxidase, barrier properties, mechanical properties, FTIR.

Introduction

Edible films can provide food protection acting as barriers to moisture, O_2 and CO_2 migration, flavor and aroma losses (Ustunol, 2009). Chitosan is a cationic polysaccharide with antimicrobial activity and excellent film-forming properties (Zhong & Xia, 2008). Chitosan has been proved to be non-toxic and biodegradable and it has been extensively used in the food industry (Coma, Martial-Gros, Garreau, Copinet, Salin & Deschamps, 2002). Chitosan films were previously demonstrated to have selective permeability to gases and good mechanical properties. The incorporation of active compounds, such as antimicrobials or antioxidants, and biocatalysts, into edible films, could avoid their direct addition to food products, and keeps them active for a longer time, retarding their degradation, and reinforcing the hurdle concept (Janjarasskul & Krochta, 2010). Due to its antimicrobial and oxygen scavenging properties, glucose oxidase (GOx), an enzyme widely used in biosensors, presents itself as an ideal candidate to be incorporated into a chitosan film or coating. GOx is a naturally produced enzyme by some fungi, namely from the genus *Aspergillus* and *Penicillium* (Wong, Wong & Chen, 2008). The reaction catalyzed by glucose oxidase consumes oxygen and produces hydrogen peroxide, a characteristic that allows GOx to be used as an antimicrobial, antioxidant and preservative in various food products. The objective of this work was to evaluate the effect of glucose oxidase incorporation in the properties of chitosan films.

Materials & Methods

Film-forming solutions of chitosan were prepared dissolving 2% (w/v) chitosan in a 1% (v/v) lactic acid solution. Glycerol (25% w/w chitosan) was added, as plasticizer and the solution was stirred until complete dispersion. To prepare the films, 30 mL of each solution were cast onto 90 mm Petri dishes and dried at 35 °C during 16h. Films were stored at 20 °C at 50% RH until further use. Chitosan films containing GOx were prepared exactly as control films, but 1.1% (v/v FFS) of GOx stock solution was added to chitosan film-forming solutions and mixed for 30 minutes before being spread onto Petri dishes.

The films' tensile strength (TS) and elongation-at-break (EB) were determined with an Instron Universal Testing Machine (model 4484; Instron Co., Canton, MA) following the guidelines of standard test ASTM D882-91, as previously described by Cerqueira, Lima, Teixeira, Moreira & Vicente (2009). Water vapour permeability (WVP) was determined gravimetrically based on ASTM E96-92 method described by Martins, Cerqueira, Souza, Avides & Vicente (2010). Oxygen (O_2P) and carbon dioxide permeability (CO_2P) were determined on the basis of the ASTM D 3985-02 method described by Cerqueira *et al*. (2009). FTIR spectra of the films were recorded using a Perkin-Elmer 16 PC spectrometer (PerkinElmer, Boston, USA) in the range from 650 – 4000 cm^{-1} using 16 scans at 1 cm^{-1} of resolution. Data analysis of each film was performed with peak fit 4.12 (SYSTAT Software Inc., Richmond, CA, USA) program. Spectra of films were deconvoluted with the second derivative method with a smoothing filter set at 20%.

Results & Discussion

Mechanical properties of the films were evaluated in terms of TS and EB which are related to structural characteristics of films (Table 1). Although Park, Daeschel & Zhao (2004) reported that the incorporation of lysozyme weakened the structure and integrity of chitosan films, with impact on mechanical properties of the films, incorporation of GOx to the chitosan matrix did not have a significant effect on film´s mechanical properties. Similar results were obtained by Souza, Fernández, López-Carballo, Gavara & Hernández-Muñoz (2010) were the incorporation

of lysozyme at 1% w/w to the polymer matrix did not have a significative effect on mechanical properties of sodium caseinate films.

Table 1. Values of tensile strength (TS), elongation-at-break (EB), water vapour permeability (WVP), O_2 permeability (O_2P) and CO_2 permeability (CO_2P) for chitosan films.

Film Type	TS (MPa)	EB (%)	WVP × 10^{-10} ($g.s^{-1}.m^{-1}.Pa^{-1}$)	O_2P × 10^{-13} ($g.s^{-1}.m^{-1}.Pa^{-1}$)	CO_2P × 10^{-14} ($g.s^{-1}.m^{-1}.Pa^{-1}$)
Chitosan	9.6 ± 1.5 [a]	65.5 ± 6.5[b]	3.16 ± 0.18 [c]	19.2 ± 1.99 [d]	4.43 ± 0.84 [e]
Chitosan + GOx	10.6 ± 1.5 [a]	71.6 ± 9.1 [b]	3.36 ± 0.32 [c]	18.5 ± 1.87 [d]	4.16 ± 0.86 [e]

Values reported are the means ± standard deviation [a-e] Different letters in the same column indicate a statistically significant difference (Tukey test $p<0.05$,).

GOx incorporation into the chitosan matrix did not change WVP values (table 1). These results are in agreement with the previous results (specially mechanical properties) in which GOx did not influence the chitosan films characteristics. The same matrix behaviour was observed by Park, Daeschel & Zhao (2004) when lysozyme was incorporated in chitosan films. Chitosan films have been reported to be good oxygen barriers but poor water barriers (Vargas, Albors, Chiralt & González-Martínez, 2009). In fact, the values obtained for O_2P and CO_2P of the films are significantly lower than those obtained for WVP. It was found that the incorporation of GOx solution 1.1% to chitosan matrix did not have a significant effect on O_2P or CO_2P.

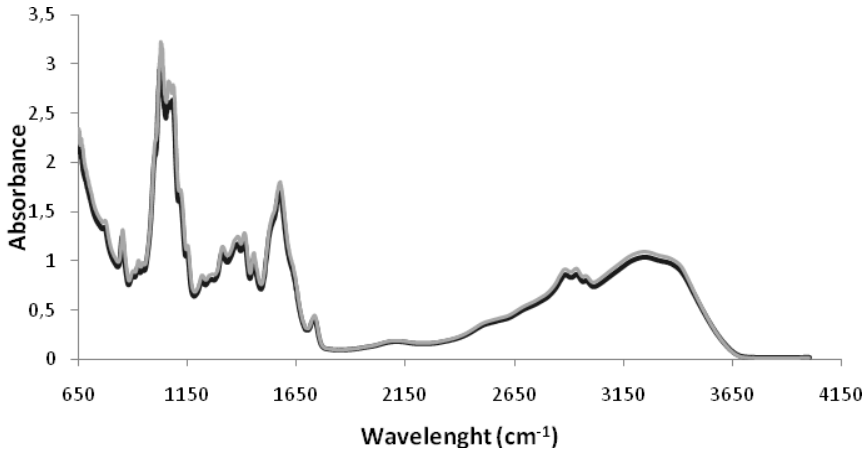

Figure 1. Spectra of FTIR for chitosan edible films; (─) chitosan film, (─) chitosan film containing GOx.

FTIR has been used to evaluate the interaction between the film and the enzyme (Figure 1). The amide I band (between 1600-1700 cm^{-1}) is one of the most useful peaks for infrared analysis of the secondary structure of proteins (Pranoto, Rakshit & Salokhe, 2005). The effect of GOx incorporation of chitosan films could be evaluated by determination of shifts in amide I band. FTIR deconvolution (data not shown) in the region 1500-1700 cm^{-1} revealed that the incorporation of GOx in the chitosan matrix, led to shifts in the amide I (1600-1700 cm^{-1}) and amide II region (1536 cm^{-1}) as well in the –NH bonds. The most significant changes were verified in amide I region (from 1621.72 to 1607.33 cm^{-1} and from 1647.27 to 1639.79 cm^{-1}). This shifts in amide I region suggest a specific chemical interaction between functional of GOx and bioactive groups of chitosan.

Conclusions

The obtained results showed that chitosan films were not significantly affected by the addition of GOx, maintaining their good mechanical and barrier properties, namely low permeability values to oxygen and carbon dioxide. FTIR analyses demonstrated the existence of specific interactions between GOx and the polysaccharide film. These results suggest that chitosan films containing GOx could be used as active packaging for extension of food's shelf-life.

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