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Effects of Interactions between the Constituents of Chitosan-Edible Films on Their Physical Properties

Miguel A. Cerqueira · Bartolomeu W. S. Souza · José A. Teixeira · António A. Vicente

Abstract The main objective of this work was to evaluate the effect of chitosan and plasticizer concentrations and oil presence on the physical and mechanical properties of edible films. The effect of the film constituents and their in-between interactions were studied through the evaluation of permeability, opacity and mechanical properties. The effects of the studied variables (concentrations of chitosan, plasticizer and oil) were analysed according to a 2^3 factorial design. Pareto charts were used to identify the most significant factors in the studied properties (water vapour, oxygen and carbon dioxide permeability; opacity; tensile strength; elongation at break and Young's modulus). When addressing the influence of the interactions between the films' constituents on the properties above, results show that chitosan and plasticizer concentrations are the most significant factors affecting most of the studied properties, while oil incorporation has shown to be of a great importance in the particular case of transport properties (gas permeability), essentially due to its hydrophobicity. Water vapour permeability values (ranging from 1.62×10^{-11} to 4.24×10^{-11} g m^{-1} s^{-1} Pa^{-1}) were half of those reported for cellophane films. Also the mechanical properties (tensile strength values from 0.43 to 13.72 MPa and elongation-at-break values from 58.62% to 166.70%) were in the range of those reported for LDPE and HDPE. Based on these results, we recommend the use of 1.5% (w/w) chitosan concentration to produce films, where the oil and plasticizer proportions will have to be adjusted in a case-by-case basis according to the use intended for the material. This work provides a useful guide to the formulation of chitosan-based film-forming solutions for food packaging applications.

Keywords Polysaccharide · Edible films · Chitosan · Physical properties

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Introduction

In recent years, food and packaging industries have been encouraged to reduce the amount of food packaging materials. This attitude is closely connected with the growing importance of the environmental issues at the consumer level, which are being translated to increasingly restrictive policies by the governments worldwide. As an answer to these issues, the commercial use of bio-based primary food packaging materials is being implemented, based on the research efforts being undertaken by many groups around the world. Related with the concerns for a safer and healthier environment, consumers also demand for food of high quality, without chemical preservatives, and with an extended shelf life, leading to increased efforts to discover, e.g. new packaging materials, natural preservatives and antimicrobials (Chien et al. 2007).

Chitosan is a natural polymer derived by deacetylation of chitin, the second most abundant biopolymer in nature after cellulose (Shahidi et al. 1999). When compared with other polysaccharides, chitosan has several important advantages such as biocompatibility, biodegradability and

no toxicity; several studies indicated chitosan as bacteriostatic and fungistatic (Kumar 2000; Möller et al. 2004; Dutta et al. 2004; Dutta and Dutta 2005).

The polycationic properties of chitosan provide the possibility of film formation by the breakage of polymer segments and subsequent reforming of the polymer chain into a film matrix or gel; this can be achieved through the evaporation of the solvent, thus creating hydrophilic and hydrogen bonding and/or electrolytic and ionic crosslinking. Chitosan proved to have some biocide properties as shown, e.g. by Fernandez-Saiz et al. (2009) and Dutta et al. (2009).

The presence of a plasticizer is often required to improve the mechanical properties of films. Common plasticizers used in edible films preparation are water, glycerol, sorbitol and other low-molecular weight polyhydroxy compounds. In particular, glycerol and sorbitol are widely used as plasticizers because of their stability and edibility (Bangyekan et al. 2006). Further, in order to improve water barrier properties, lipids are frequently incorporated into hydrocolloid-based films (Fabra et al. 2008).

The main objective of this work was to evaluate the effect of the concentrations of chitosan, plasticizer and oil in relevant properties of chitosan-based films. The influence of the interactions between those constituents was evaluated in terms of differences observed in water vapour permeability, oxygen permeability, carbon dioxide permeability, opacity, tensile strength, elongation at break and Young's modulus.

Material and Methods

Materials

Edible coating solutions were prepared using chitosan with a degree of deacetylation of 90%, approximately (Aqua Premier Co., Thailand), corn oil (Sovena, Portugal), glycerol 87% (Panreac, Spain), sorbitol 97% (Acros Organics, Belgium), Tween 80 (Acros Organics, Belgium), lactic acid (Merck, Germany) and distilled water.

Experimental Design

Two sets of experiments (experimental setups I and II) were established for the formulation of film-forming solutions, where the main difference was the type of plasticizer used: glycerol alone (setup I) or a 50:50 mixture of glycerol and sorbitol (setup II). Then, a 2^3 experimental design was applied to each experimental setup, as displayed in Table 1. The independent variables were chitosan, glycerol and oil in the experimental setup I and chitosan, glycerol/sorbitol mixture and oil in the experimental setup II. Concentrations levels as presented in Table 1 were chosen based on preliminary experiments (data not shown) where their

Table 1 Factors and levels used to analyse chitosan properties in experimental setups I and II

	Factors	Levels	
Experimental setup I	Chitosan	0.5	1.5
	Glycerol	0.5	2.0
	Oil	0.0	0.5
Experimental setup II	Chitosan	0.5	1.5
	Glycerol/sorbitol	0.5	2.0
	Oil	0.0	0.5

suitability for film formation was tested. Materials present good miscibility at the concentrations used.

Films Preparation

The film-forming solutions (see Table 2) were prepared by dissolving chitosan in a 1.0% (v/v) lactic acid solution with agitation using a magnetic stirrer (at 200 rpm) during 2 h at room temperature (20 °C); Tween 80 was also added as a surfactant at a concentration of 0.2% (w/v), together with glycerol or a mixture of glycerol and sorbitol (50:50), which were used as plasticizers. When pertinent (Table 2) corn oil was subsequently added in a concentration of 0.5% (w/v), under agitation during 20 min at 60 °C. To produce the films, a constant amount (13 mL) of film forming solution was cast onto a 5.7 cm diameter glass plate. The films were dried in an oven at 35 °C during 16 h. Films were maintained at 20 °C and 50% RH (average laboratory conditions) before permeability and opacity tests.

Film Thickness

The film thickness was measured with a digital micrometer (No. 293–561, Mitutoyo, Japan). Five thickness measurements were taken on each testing sample in different points and the mean values were used to calculate permeability and mechanical values.

Water Vapour Permeability Measurement

The measurement of water vapour permeability measurement (WVP) was performed gravimetrically based on ASTM E96-92 method (McHugh et al. 1993; Guillard et al. 2003). The film was sealed on the top of a permeation cell containing distilled water (100% RH; 2,337 Pa vapour pressure at 20 °C), placed in a desiccator at 20 °C and 0% RH (0 Pa water vapour pressure) containing silica. The cells were weighted at 2-h intervals during 10 h. Steady-state and uniform water pressure conditions were assumed by keeping the air circulation constant outside the test cell by using a miniature fan inside the desiccator (McHugh et al. 1993). The slope of

Table 2 Chitosan, plasticizer and oil concentrations used in films formulation

Sample	Chitosan (w/v)	Glycerol (w/v)	Glycerol/sorbitol (w/v)	Oil (w/v)
1	0.5	0.5		
2	0.5	2.0		
3	0.5	0.5		0.5
4	0.5	2.0		0.5
5	0.5		0.5	
6	0.5		2.0	
7	0.5		0.5	0.5
8	0.5		2.0	0.5
9	1.5	0.5		
10	1.5	2.0		
11	1.5	0.5		0.5
12	1.5	2.0		0.5
13	1.5		0.5	
14	1.5		2.0	
15	1.5		0.5	0.5
16	1.5		2.0	0.5

weight loss versus time was obtained by linear regression. Three replicates were obtained for each film.

Oxygen and Carbon Dioxide Permeability

Oxygen permeability (O_2P) and carbon dioxide permeability (CO_2P) were determined based on the ASTM D 3985–02 (2002) method. The films were sealed between two chambers, having each one two channels. In the lower chamber, O_2 (or CO_2) was supplied at a controlled flow rate (J & W Scientific, ADM 2000, USA) to keep its pressure constant in that compartment. The other chamber was purged by a stream of nitrogen, also at controlled flow. Nitrogen acted as a carrier for the O_2 (or the CO_2).

In the case of O_2P measurement, the flow leaving this chamber was connected to an O_2 sensor which measured the O_2 concentration in that flow online. In the case of CO_2P measurement, the flow leaving this chamber was collected with a syringe for CO_2 quantification. To determine CO_2 concentration, 1 mL of sample (from the syringe) was injected in a gas chromatograph (Chrompack 9001, Middelburg, The Netherlands) at 110 °C with a column Porapak Q 80/100 mesh 2 m×1/8″×2 mm SS, using a flame ionisation detector at 110 °C. Helium at 23 mL min^{-1} was used as carrier gas. A standard mixture containing 10% CO_2, 20% O_2 and 70% N_2 was used for calibration. The pressure in the cells was measured with an inline manometer to ensure that 1 atm pressure exerted in both cells. As the O_2 (and the CO_2) was carried continuously by the nitrogen flow, it was considered that O_2 (and the CO_2) partial pressure in the upper compartment is null, therefore ΔP is equal to 1 atm. Three replicates were obtained for each sample, in each case (O_2P and CO_2P)

Opacity

The opacity of films was determined with a Minolta colorimeter (CR 400; Minolta, Japan). The opacity of the samples was determined according to the Hunter lab method, as the relationship between the opacity of each sample on the black standard (Y_b) and the opacity of each sample on the white standard (Y_w).

Tensile Strength, Elongation at break, Young's Modulus

Tensile strength (TS), elongation at break (EB) and Young's modulus (YM) were measured with an Instron Universal Testing Machine (Model 4500, Instron Corporation) following the guidelines of ASTM D 882–91 (1991) method. The initial grip separation was set at 30 mm and the crosshead speed was set at 5 mm/min. TS was expressed in megapascal and calculated by dividing the maximum load (N) by the initial cross-sectional area (m^2) of the specimen. EB was calculated as the ratio of the final length at the point of sample rupture to the initial length of a specimen (30 mm) and expressed as a percentage. According to the ASTM standard, film strips with a length of 45 mm and a width of 20 mm were used. TS, EB and YM tests were replicated at least five times for each type of film.

Statistical Analysis

Data analyses were performed using Statistica software (release 7, edition 2004, Statsoft, Tulsa, OK, USA). Pareto charts were drawn to express visually the statistical significance of each factor and the interactions between factors.

The experimental data were fitted to a multifactor model, represented by Eq. 1:

$$Y = a + b \cdot X_1 + c \cdot X_2 + d \cdot X_3 + e \cdot X_1 \cdot X_2 + f \cdot X_1 \\ \cdot X_3 + g \cdot X_2 \cdot X_3 \tag{1}$$

where, Y represents the dependent variables: WVP, O_2P, CO_2P, opacity, TS, EB or YM; and X_n are the independent variables: chitosan ($n=1$), glycerol ($n=2$) and oil ($n=3$) in the experimental setup I; being the dependent variables represented in experimental setup II by Y' where the independent variables are: chitosan ($n=1$), glycerol/sorbitol ($n=2$) and oil ($n=3$).

The fitting accuracy of the models was evaluated by the determination of the following parameters: coefficient of determination (R^2), mean relative deviation modulus (E) and accuracy factor (A_f). The goodness of fit was quantified by R^2 which provides the percentage of the variance of the data that is explained by the model:

$$R^2 = 1 - \frac{SSR}{SSD} \tag{2}$$

The higher the R^2 value, the better the model fits the experimental data (Neter et al. 1996). The mean relative percentage deviation modulus, E, in Eq. 3, indicates the goodness of the fit between the observed and predicted values of the analysed parameters for the independent variables used, N being the number of data points, R_{obs} the observed values of each parameter, and R_{pre} the values predicted by the model. Values below 10% are indicative of a good fit (McLaughlin and O'Beirne 1999).

$$E = \frac{100}{N} \cdot \sum_{1}^{N} \frac{\left| (R_{obs} - R_{pre}) \right|}{R_{obs}} \tag{3}$$

The accuracy factor (A_f) also provides information on the fitting accuracy. The closer the A_f value is to 1, the better the accuracy (Ross 1996):

$$A_f = 10 \cdot {\left(\sum_{j=1}^{j=J} \frac{\left| \log(\frac{R_{pred}}{R_{obs}}) \right|}{J} \right)} \quad j = 1, 2, 3, \ldots, \tag{4}$$

J = number of observations

Results and Discussion

Water Vapour Permeability

The water vapour permeability is the most extensively studied property of edible films mainly because of the importance of water in deteriorative reactions in foods. In

Fig. 1a.I, a.II Pareto charts show that oil is one of the most important factors influencing the value of WVP. Oil and plasticizer are the factors with more significance, only overtaken in experimental setup II by chitosan. In experimental setup I, chitosan has not a significant effect in WVP. Table 3 shows that WVP increases with the increase of plasticizer concentration; this observation is common to both experimental setups. Higher concentrations of plasticizer favour the adsorption of water molecules, which is mainly attributed to the predisposition of plasticizers to form hydrogen bonds. Plasticizers and its plasticizing action change the polymer network creating mobile regions with greater interchain distances, promoting water clustering by competing with water at active sites of the polymer matrix and reducing intermolecular hydrogen bonding between chitosan molecules (Diab et al. 2001; Olivas and Barbosa-Cánovas 2008). Similar results were also shown by Caner et al. (1998) and Ziani et al. (2008), where the increase of the concentration of plasticizer has increased the values of WVP; the same being quantified in starch-based films (Mali et al. 2006) and pullunan films (Diab et al. 2001). Results show that the incorporation of sorbitol instead of glycerol is an alternative to reduce the effect of glycerol in the increase of WVP in chitosan films, since in some cases the values of WVP are lower (Table 3). These results may be explained by the free volume theory: plasticizers increase the free volume of polymer structures or the molecular mobility of polymer molecules. In the present case, the decrease of WVP may be attributed to the larger size and relatively lower hygroscopicity of sorbitol compared to glycerol that might reduce the amount of water entrapped in the film matrix; such film would therefore have a higher effective concentration of polysaccharide, thus reducing water mobility and, consequently, reducing WVP. Previous works have shown that edible films containing sorbitol as plasticizer have lower WVP values than those containing glycerol (Olivas and Barbosa-Cánovas 2008; Chick and Ustunol 1998; Fairley et al. 1996), probably due to sorbitol being less effective than glycerol in reducing intermolecular hydrogen bonding between chitosan molecules.

For all the films, the incorporation of oil decreased the WVP as the presence of the hydrophobic oil blended with chitosan changes the film properties decreasing the water affinity (Table 3). Similarly, Wong et al. (1992) observed a higher resistance to water vapour transmission when lauric and butyric acids were used in chitosan films and Park and Zhao (2004) incorporated vitamin E into the chitosan matrix obtaining a significant decrease in WVP. Recently, Vargas et al. (2009) showed that the addition and further increases of oleic acid in chitosan films decreased the WVP values. The decreasing of the WVP with the addition of oil can be explained by the reduction of the hydrophilic portion

of the film (Hernandez-Munõz et al. 2004). In the present work, the lowest values of WVP were obtained for the films made with a mixture of 1.5% of chitosan with 0.5% of oil and 0.5% of glycerol/sorbitol (4.04×10^{-11} g Pa^{-1} s^{-1} m^{-1}). These values are in agreement with those obtained in other works (Table 4). Chitosan films evaluated in this work presented WVP values in the range of cellophane films.

When the model equation (Eq. 1) was fitted to the WVP experimental data a good fit, with R^2 above 0.88, E below 10% and A_f very close to 1, was obtained in all cases (see Table 5).

Oxygen Permeability

Oxygen is the key factor that might cause oxidation and which initiates several unwanted changes in foods such as odour, colour, flavour and nutrients deterioration. Films providing a proper barrier to oxygen can help improving food quality and extending food shelf life. Chitosan and plasticizer concentrations are the most significant factors in O_2P, as shown by Pareto charts (Fig. 1b.I, b.II). Table 3 shows that the increase of plasticizer concentration provoked a significant decrease in the O_2P values. In this case, the increase of the number of
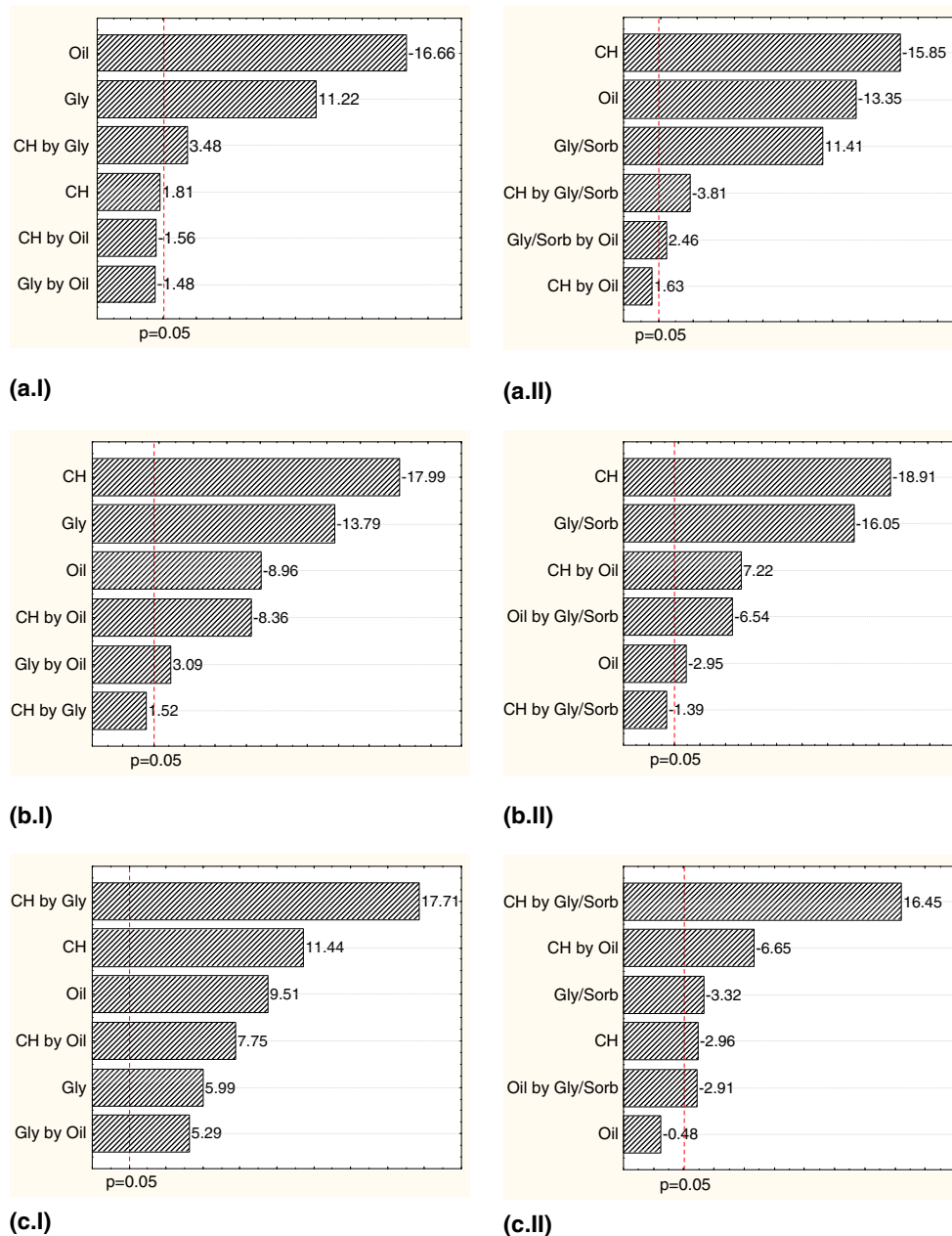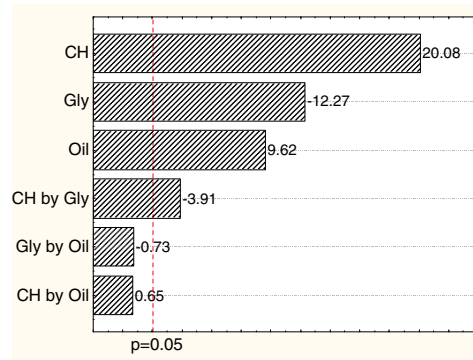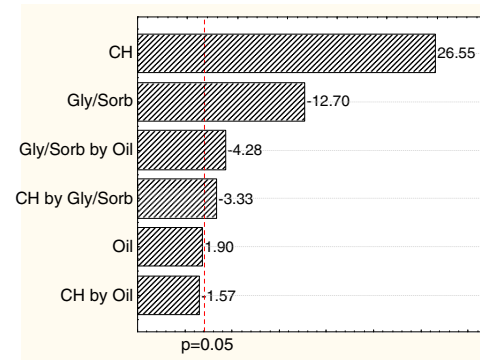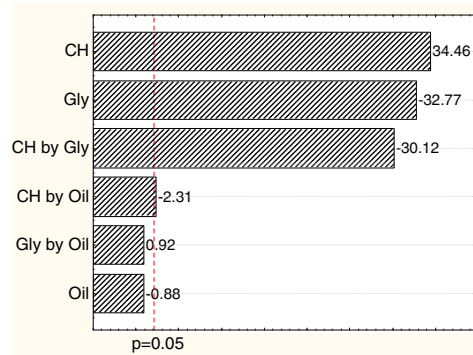

Fig. 1 Pareto charts for the experimental setup I (*I*) and the experimental setup II (*II*): water vapour permeability (*a*), oxygen permeability (*b*), carbon dioxide permeability (*c*), opacity (*d*), tensile strength (*e*), elongation at break (*f*) and Young's modulus (*g*)
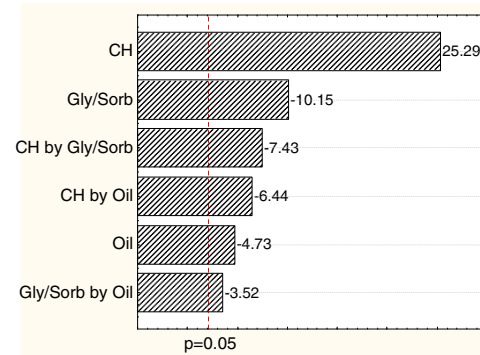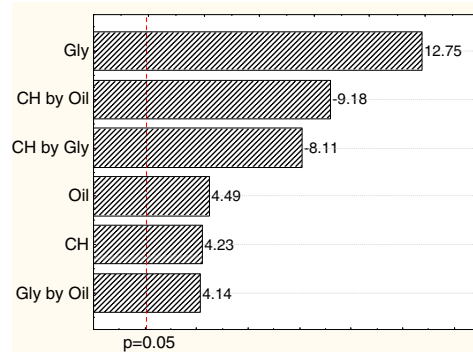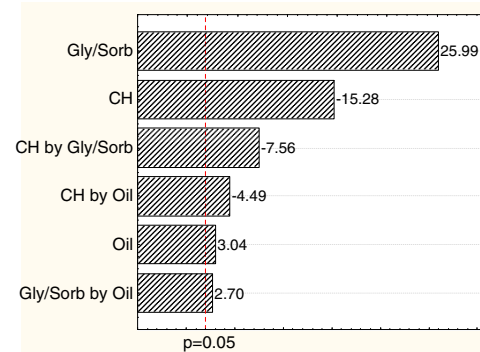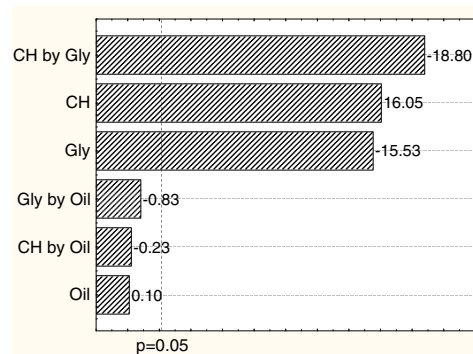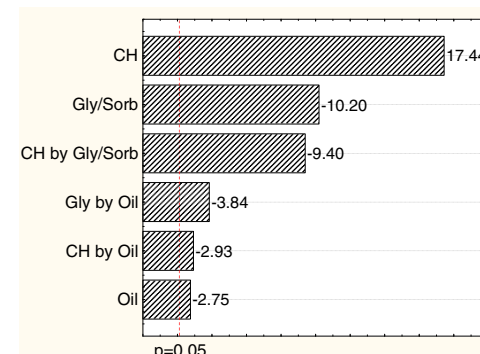
(d.I)

(d.II)

(e.I)

(e.II)

(f.I)

(f.II)

(g.I)

(g.II)

Fig. 1 (continued)

Table 3 Values of thickness, water vapour permeability (WVP), oxygen permeability (O_2P), carbon dioxide permeability (CO_2P), and permeaselectivity (CO_2P/O_2P) for each film's formulation

Sample	Thickness (mm)	WVP×10^{11} (g Pa^{-1} s^{-1} m^{-1})	O_2P×10^{-15} (g Pa^{-1} s^{-1} m^{-1})	CO_2P×10^{-15} (g Pa^{-1} s^{-1} m^{-1})	CO_2P/O_2P
1	0.060±0.004 a, c	8.19±0.55 a	7.61±0.38 a	35.65±1.99 a,c	4.82
2	0.062±0.003 a,b	10.30±0.79 b	5.15±0.55 b	23.42±2.92 b	4.55
3	0.059±0.002 a	6.87±0.29 c	7.46±0.49 a	36.41±3.52 a, c	4.88
4	0.067±0.002 b	7.08±0.22 c, d	5.11±0.09 b	27.53±3.39 b	5.39
5	0.060±0.004 a, c	7.65±0.48 a, d	8.96±0.51 c	31.32±3.06 a	3.49
6	0.056±0.001 c, d	8.36±0.33 a, e	7.45±0.86 a, e	30.21±3.82 a, b	4.06
7	0.052±0.003 d	6.26±0.35 c	9.19±0.54 c	36.15±2.72 c	3.93
8	0.053±0.002 d	7.68±0.28 a	4.73±0.58 d	18.78±2.41 d	3.97
9	0.067±0.001 b	8.90±0.47 e	6.29±0.52 e	26.68±1.75 b	4.24
10	0.066±0.001 b	10.60±0.09 b	3.46±0.62 f	39.84±1.71 e	11.51
11	0.063±0.001 a	5.65±0.62 f	2.51±0.51 f	34.63±3.61 a, c	13.80
12	0.060±0.002 a	8.37±0.39 a	1.65±0.53 g	59.80±3.42 f	36.24
13	0.071±0.005 b	6.08±0.36 f	5.59±0.41 b	16.00±0.55 d	2.86
14	0.067±0.004 a, b	7.32±0.48 a	2.88±0.64 f	34.36±5.95 a, c	11.93
15	0.053±0.001 d	4.04±0.10 g	6.58±0.23 e	15.66±1.24 d, g	2.38
16	0.055±0.001 c, d	5.63±0.21 f	3.35±0.68 f	21.24±3.55 g	6.34

Means in the same column with different letters are significantly different ($p<0.05$).

hydroxyl groups due to a higher plasticizer concentration enhanced the effect of hydrogen bounding and increased the cohesive energy density. The cohesive energy density is a measure of the polarity of a polymer and of the energy binding the polymer chains together. In general, the higher the polymer cohesive energy density, the more difficult it is for the polymer chains to open and allow a permeant to pass (polar permeants such as water being an exception to this rule; Miller and Krochta 1997).

The lowest value of O_2P was obtained with the film made with 1.5% of chitosan, 2.0% of glycerol and 0.5% of oil ($1.65×10^{15}$ g Pa^{-1} s^{-1} m^{-1}). The obtained values

are higher than those reported by Caner et al. (1998) where a lower amount of plasticizer was added (Table 4). The chitosan films evaluated in this work presented oxygen permeability values in the range of cellophane films.

The model fitted to O_2P data (Table 5) presents an acceptable value for R^2 (above 0.93) and a value of A_f close to 1; however, the value of E range between 10% and 20% in both cases, therefore indicating that in this case the fit between the observed and predicted values is not as close as for the other variables tested in this work (Table 5).

Table 4 Values of water vapour permeability (WVP), oxygen permeability (O_2P), tensile strength (TS), elongation at break (EB) and Young's modulus (YM) reported in the literature for films

Film composition	WVP×10^{-11} (g Pa^{-1} s^{-1} m^{-1})	O_2P×10^{-15} (g Pa^{-1} s^{-1} m^{-1})	TS (MPa)	EB (%)	YM (MPa)	References
1% Chitosan	4.5		60.7	3.3		Garcia et al. (2006)
1% Chitosan	3.7					Wong et al. (1992)
3% Chitosan	9.49	0.12	22.67	32.19		Caner et al. (1998)
1% Chitosan	24.72		60.0	42.1		Ziani et al. (2008)
1% chitosan–0.2% glycerol	30.83		47.1	67.3		Ziani et al. (2008)
2% Chitosan			51.2	4.8	34.1	Altiok et al. (2010)
Cellophane	8.4		85.8	14.4		Garcia et al. (2006)
LDPE	0.0914		16.2	68.7		Garcia et al. (2006)
HDPE	0.023		27.8	150		Garcia et al. (2006)
LDPE					140	Tai et al. (2000)
HDPE					1,490	Tai et al. (2000)

Table 5 Model equations and the corresponding quality of the fit evaluation parameters

	Model equations	R^2	E	A_f
Experimental setup I	WVP$_I$ = 8.2713 − 0.3671·x_1 + 0.5764·x_2 − 3.3336·x_3 + 0.6995·x_1·x_2 − 0.9412·x_1·x_3 − 0.5957·x_2·x_3	0.88	5.20	1.05
	O$_2$P$_I$ = 9.73080 − 1.9027·x_1 − 2.0779·x_2 + 0.8177·x_3 + 0.3164·x_1·x_2 − 5.2328·x_1·x_3 + 1.2885·x_2·x_3	0.95	6.37	1.07
	CO$_2$P$_I$ = 52.1856 − 20.8821·x_1 − 18.7193·x_2 − 23.9486·x_3 + 19.0938·x_1·x_2 + 25.0603·x_1·x_3 + 11.4097·x_2·x_3	0.95	3.14	1.03
	Opacity$_I$ = 2.2498 + 1.1545·x_1 − 0.1100·x_2 + 0.8488·x_3 − 0.2321·x_1·x_2 + 0.1151·x_1·x_3 − 0.0868·x_2·x_3	0.95	2.61	0.98
	TS$_I$ = −7.4056 + 16.7415·x_1 + 3.4071·x_2 + 0.8487·oil − 7.7304·x_1·x_2 − 1.7801·x_1·oil + 0.4729·x_2·x_3	0.99	17.97	1.26
	EB$_{(I)}$ = 21.6055 + 47.4768·x_1 + 29.2000·x_2 + 56.2840·x_3 − 19.0687·x_1·x_2 − 64.7540·x_1·x_3 + 19.4520·Gly·x_3	0.89	5.72	1.06
	YM$_{(I)}$ = 7.4938 + 9.5229·x_1 − 9.2163·x_2 + 0.0611·x_3 − 11.1516·x_1·x_3 − 0.1379·x_1·x_2 − 0.4939·x_2·x_3	0.98	>20	0.56
Experimental setup II	WVP$_{II}$ = 8.1478 − 1.6040·x_1 + 0.4111·x_2 − 2.1366·oil + 0.2368·x_1·x_2 − 1.6551·x_1·oil + 0.7134·x_2·x_3	0.95	1.30	1.01
	O$_2$P$_{II}$ = 11.8731 − 4.0574·x_1 − 0.7635·x_2 − 2.2079·x_3 − 0.3168·x_1·x_2 + 4.9187·x_1·x_3 − 2.9722·x_2·x_3	0.93	8.40	1.09
	CO$_2$P$_{II}$ = 45.6805 − 21.8885·x_1 − 20.4924·x_2 + 32.6308·x_3 + 20.2395·x_1·x_2 − 24.5676·x_1·x_3 − 7.1642·x_2·x_3	0.88	8.99	1.09
	Opacity$_{II}$ = 2.5467 + 1.1261·x_1 − 0.0394·oil + 0.8146·oil − 0.1484·x_1·x_2 − 0.2100·x_1·x_3 − 0.3819·x_2·x_3	0.96	2.56	0.98
	TS$_{II}$ = −6.5835 + 13.6604·x_1 + 1.7006·x_2 + 8.6811·x_3 − 3.0689·x_1·x_2 − 7.9805·x_1·x_3 − 2.9042·x_2·x_3	0.98	>20	1.91
	EB$_{(II)}$ = 56.6677 + 4.4807·x_1 + 63.6467·x_2 + 37.3700·x_3 − 25.0533·x_1·x_2 − 44.5960·x_1·x_3 + 17.8880·x_2·x_3	0.95	5.54	1.06
	YM$_{(II)}$ = 9.0125 + 15.7016·x_1 − 9.1809·x_2 − 2.4775·x_3 − 8.4596·x_1·x_2 − 2.6342·x_1·x_3 − 3.4598·x_2·x_3	0.96	>20	0.22

Carbon Dioxide Permeability

Carbon dioxide concentration is very important in specific modified atmosphere packaging applications. This is true for non-respiring foods (e.g. fish and meat) and also for respiring foods (e.g. fruits) if considered together with O$_2$P (the concept of *permselectivity* is often considered in this case). In the case of CO$_2$P, the interaction between chitosan and glycerol and chitosan alone in experimental setup I and the interaction of chitosan and glycerol/sorbitol as well as the interaction of chitosan and oil in the experimental setup II are the most significant factors affecting the value of CO$_2$P (Fig. 1c.I, c.II). These results show that plasticizers have different influences on the values of O$_2$P and CO$_2$P. The plasticizers increase the cohesive energy density and the free volume of polymer structures. The influence of the cohesive energy density in the values of O$_2$P has been previously discussed and it can also explain the decrease of the CO$_2$P values for concentrations of 0.5% of chitosan. For higher concentrations of chitosan (1.5%), the free volume of polymer seems to be the most important factor, leading to an increase of CO$_2$P with the increase of the plasticizer concentration (Table 3). These observations are in line with those of Miller and Krochta (1997), who reported that the diffusion coefficient and the permeability coefficient both increase with the increase in free volume for carbon dioxide in various polymers. The higher values obtained for CO$_2$P when compared with the oxygen permeability can be explained by the solubility of these gases in water. The CO$_2$ is approximately 35 times more soluble than O$_2$ in water, and this is the reason why this gas diffuses much faster, therefore increasing its permeability as observed in this study at 50% RH (Mujica-Paz and Gontard 1997).

Table 3 shows different behaviours of CO$_2$P with the increase of the plasticizer concentrations in the presence of two chitosan concentrations. In both experimental setups (I and II), the values of CO$_2$P decrease when the plasticizer concentration is increased for chitosan concentrations of 0.5%, while in the presence of a concentration of 1.5% of chitosan, the increase of plasticizer concentration leads to an increase of CO$_2$P. The lowest value of CO$_2$P was obtained for films containing 1.5% of chitosan and 2.0% of glycerol 59.80×10^{15} g Pa^{-1} s^{-1} m^{-1}.

The permselectivity, that is the ratio between CO$_2$ and O$_2$ permeabilities (CO$_2$P/O$_2$P), has been calculated due to its implications when designing packaging films for food applications (Park and Chinnan 1995; Al-Ati and Hotchkiss 2003; Wu et al. 2009). Permselectivity is commonly used as an indication of the appropriateness of a given film material for a specific food packaging application. Commercial packaging films have typical CO$_2$P/O$_2$P values ranging

between 4 and 8 (Al-Ati and Hotchkiss 2003). A higher ratio indicates a lower accumulation of CO_2 and vice versa. Chitosan permselectivity values ranged between 2.38 and 36.24, for 1.5% of chitosan, 0.5% of glycerol/sorbitol and 0.5% oil, and 1.5% of chitosan, 2.0% of glycerol and 0.5% of oil, respectively (Table 3). Such a wide range of permselectivity values allows the use of chitosan films in different food packaging applications, however for highly respiring food products (e.g. mushrooms, strawberries, fresh-cut vegetables) lower values of permselectivities are needed (Exama et al. 1993). The fitting of the model equation (Eq. 1) to the experimental values of CO_2P shows quite good results, with values of R^2 above 0.88, E below 10% and A_f very close to 1 (Table 5).

Opacity

The opacity is important to control the incidence of light on a food product, being a relevant property since it has a direct impact on the appearance of the food product. Chitosan concentration is the most important factor affecting the opacity of chitosan-based films, followed by the plasticizer concentration (Fig. 1d.I, d.II). The increase of chitosan concentration originates a film matrix with a stronger polymer network resulting on higher values of opacity; on the other hand, the increase of glycerol concentration leads to an increase of the free volume of the polymer network, as explained elsewhere, thus increasing the mobility of the polymer chains and decreasing the opacity by permitting a better penetration of the light. Table 5 shows that the values of opacity are higher in films containing oil.

The increase of opacity registered when oil was added was probably a result from the presence of lipid droplets formed during the coating formulation that are dispersed in the emulsion and distributed in the polymer matrix after the film is formed. Yang and Paulson (2000) demonstrated that also gellan films have an increased opacity with the increase of lipid concentration. Park and Zhao (2004) incorporated vitamin E into a chitosan matrix obtaining a significant increase of opacity. The fitting of the model equation (Eq. 1) to the experimental values of opacity shows very good results, with values of R^2 above 0.95, E below 10% and an A_f value of 0.98 (Table 5).

Tensile Strength

Tensile strength is the ability of a material to resist breaking under tensile stress and is one of the most important and widely measured properties of materials used in structural applications. The most significant factors affecting TS in the studied films (Fig. 1e.I, e.II) were chitosan concentration, glycerol concentration and the interaction between both. The increase of chitosan concentration increased the tensile strength values (Table 6). The increase of chitosan concentration leads to a stronger gel network, where the polysaccharide molecules are closer, forming a more coherent film structure, and reducing the absorption of water molecules. This behaviour is in agreement with the work presented by Casariego et al. (2009), where the increase of the chitosan concentration led to an increase of TS. The increase of plasticizer concentration has great influence on the values of TS for films containing 1.5% chitosan, leading to a decrease of that value of approximately 90% (Table 6). This behaviour is explained by the plasticizing effect of glycerol and sorbitol that changes the polymer network and creates more mobile regions with

Table 6 Values of opacity, tensile strength (TS), elongation at break (EB) and Young's modulus (YM) for each film's formulation

Sample	Opacity	TS (MPa)	EB (%)	YM (MPa)
1	2.74±0.20 a	0.63±0.01 a	58.62±7.83 a	1.52±0.25 a
2	2.44±0.13 b	0.15±0.01 b	81.24±7.53 b	3.75±1.43 b, d
3	3.22±0.33 c	0.94±0.17 c	68.55±6.53 a	2.01±0.39 a, d
4	2.78±0.32 b	0.39±0.04 d	119.52±4.45 c	3.65±2.68 a, b
5	3.03±0.21 b	0.70±0.11 c	78.92±9.75 b	0.79±0.11 c
6	3.85±0.38 b	0.21±0.05 b	166.70±7.36 d	1.38±0.80 a, c
7	2.28±0.26 b	1.58±0.14 e	102.03±4.63 e	2.26±0.26 d
8	2.85±0.22 b	0.43±0.06 d	181.02±5.21 d	0.22±0.07 e
9	3.94±0.44 qd	13.72±0.43 f	89.68±4.99 b	22.13±1.88 f
10	2.99±0.12 e	1.22±0.20 e	97.45±3.77 e	2.46±0.32 d
11	4.35±0.48 d	12.71±1.22 f	81.00±4.99 b	22.75±1.94 f
12	3.60±0.20 c	0.99±0.10 c	89.60±2.05 b	1.69±0.18 a
13	4.08±0.17 b	12.08±0.58 f	81.97±8.77 b	25.44±0.40 f
14	3.75±0.18 b	8.47±0.61 g	109.97±9.83 e	13.40±1.42 g
15	4.58±0.49 b	10.46±1.92 g	60.58±6.74 a	25.93±6.0 f
16	3.55±0.15 b	3.18±0.43 h	124.20±8.79 c	2.67±0.07 d

Means in the same column with different letters are significantly different ($p<0.05$)

larger interchain distances, thus decreasing TS. Also, Caner et al. (1998) showed that the increase of plasticizer concentration decreases the value of TS and Ziani et al. (2008) obtained similar results with glycerol as plasticizer (Table 4). In our work, the highest values of tensile strength were obtained for 1.5% of chitosan and 0.5% of glycerol (13.72 MPa). The obtained values are in the range of the TS values for low-density polyethylene films (Table 4).

The fitting of the model equation (Eq. 1) to the experimental values of TS shows a good value of R^2 above 0.98; however, the values of A_f and E present values distant from the optimum (Table 5) indicating that the fit between the observed and predicted values is not as close as for the other variables tested in the present work.

Elongation at Break

The elongation at break of an engineering material is the percentage increase in length that occurs before it breaks under tension. Ultimate elongation values of several hundred percent are common for elastomers and film/packaging polyolefins. All of the studied factors in this work have significant influence in elongation (Fig. 1f.I, f.II), but the most significant is the plasticizer concentration: the increase of the plasticizer concentration leads to an increase of EB. Plasticizers interfere with chitosan chains and by decreasing intermolecular forces, soften the rigidity of the film structure and increase the polymer mobility, which facilitates film elongation (Srinivasa et al. 2007). These results are in agreement with those of Caner et al. (1998) that show an increase of elongation values with the increase of plasticizer concentration. Also Ziani et al. (2008) showed that the increase of glycerol concentration leads to an increase of the EB (Table 4). A value of EB of 67.3% was obtained for films of 1.0% chitosan with 0.2% glycerol. In the present work, the value of EB for the film formulation containing 1.5% of chitosan and 0.5% of glycerol was 89.68%. The higher value in our work when compared with the work of Ziani et al. (2008) could be explained by the lower ratio chitosan/glycerol used in the later: (3:1) and (5:1), respectively. The chitosan films evaluated in this work have elongation-at-break values in the range of cellophane films (Table 4). The fitting of the model equation (Eq. 1) to the experimental values of EB shows quite good results, with values of R^2 above 0.89, E below 10% and A_f very close to 1 in both cases (Table 4).

Young's Modulus

Polymer and plasticizer concentration are the most influential factors on Young's modulus values (Fig. 1g), much in the same way it happened for TS and EB. For films of chitosan with glycerol as plasticizer, the addition of oil does not have a statistically significant effect on the values of YM (Fig. 1gI). The highest values of YM where obtained for chitosan concentrations of 1.5% and 0.5% of plasticizer (Table 6). Films with the highest concentration of chitosan (1.5%) and the lowest concentrations of plasticizer (0.5%) show a higher YM, thus indicating the occurrence of a more coherent film structure with a stronger gel network, where the polysaccharide molecules are more closely packed.

The fitting of the model equation (Eq. 1) to the experimental values of YM shows a good value of R^2 above 0.96; however, the values of A_f and E are farther from the optimum (Table 6) indicating that the fit between the observed and predicted values is not as close as for the other variables tested in the present work.

Conclusion

This work shows how different chitosan-based films formulations can change film properties (permeability, mechanical and optical properties). Chitosan and plasticizer concentrations are the most significant factors in these films properties, while oil incorporation has shown to be of a great importance in the permeability properties, essentially due to its hydrophobicity. The chitosan films evaluated in this work are a good oxygen barrier and have water vapour permeability and elongation at break values in the range of, e.g. cellophane films. Chitosan films also show tensile strength values close to those reported for protein films and, for some of our films formulations, in the range of, e.g. low-density polyethylene.

Based on these results, we recommend the use of 1.5% (w/w) chitosan concentration to produce films, where the oil and plasticizer proportions will have to be adjusted in a case-by-case basis according to the use intended for the material (e.g. cheese, fish, tomato and carrots; see Cerqueira et al. 2010; El Ghaouth et al. 1991; Souza et al. 2010). This work provides important information for chitosan-based films formulation as biodegradable and edible packaging materials.

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