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Microstructural characterization of chitosan films used as support for ferulic acid release

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ABSTRACT

The influence of natural antioxidants incorporated to biodegradable materials has become a focus of attention in the current food packaging research and development. Chitosan is a functional natural polymer extensively used for tailoring systems or matrices for a different active compound delivery. This work was focused on studying the changes undergone by the chitosan matrix because of the addition of ferulic acid as an antioxidant. Thus, both microstructure and physical properties such as solubility, thermal stability, mechanical and barrier properties were monitored. The addition of ferulic acid caused a decrease in both the moisture content and water vapor permeability, an increase in resistance and a change at the structural level evidenced by TEM. Through FTIR spectra and their relationship with chitosan-based film properties, it was demonstrated that ferulic acid was effectively incorporated in the polymer matrix. The amount of the bioactive compound released from the chitosan matrix to a liquid medium was determined. The delivery profile suggested that the release of the antioxidant agent was controlled by two parallel mechanisms, one Fickian-type and the other associated to the high swelling of the matrix. The antioxidant and UV-barrier properties induced by the addition of ferulic acid turned the chitosan films into a potentially active material to be applied on high-fat foods.

Keywords: Bioactive films; chitosan; controlled release; ferulic acid.

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Introduction

Nowadays, the use of natural polymers represents a promising alternative as biodegradable and renewable materials. Chitosan is a functional natural polymer extensively used for tailoring systems or matrices for different active compounds delivery and with high potential to be used as a biodegradable active packaging. A wide number of studies have been conducted on chitosan applications reporting its uses in the pharmaceutical, medical and food fields **[1-3]**.

Ferulic acid (FA) is a ubiquitous plant constituent that arises from the metabolism of phenylalanine and tyrosine. This bioactive compound has recognized antioxidant

activity associated with the hydroxyl groups present in its structure that can possibly contribute to its free radical scavenging capability **[4-8]**. Several authors have studied the ferulic acids and their derivatives in order to improve the water solubility as well as antioxidant activity of polymeric matrices **[9, 10]**.

The design and application of biopolymers as supports for transference, regulation and controlled release of components is currently one of the most important aspects in the development of active materials. These systems consist in adding active compounds to a polymeric matrix, which acts as an efficient carrier **[11, 12]**. Antioxidant package is an emerging technology in which the antioxidant agents are incorporated into food packaging materials. Antioxidants are widely used as food additives to improve the oxidative stability of lipids and to prolong shelf life, mainly for dried products and O_2-sensitive foods.

In this way, several researchers reported the release of compounds with antioxidant properties from packaging materials. Wessling et al. **[13, 14]** studied the diffusion of butylhydroxytoluene (BHT) and α–tocopherol from low density polyethylene (LDPE) and polypropylene (PP) to food's simulant. It is well known that BHT has a disadvantage in terms of its toxicity level causing a health risk to the consumers. Currently, the tendency to reduce the use of synthetic additives in packaging has been focused on replacing them with natural derivatives.

The influence of natural antioxidants incorporated to biodegradable material has become a focus of attention in the current food packaging research and development **[4, 5, 15]** thus, more research is needed. As far as we know, there is hardly any study discussing the ferulic acid delivery for preservation in the food area.

Taking into consideration that antioxidant activity depends mainly on the diffusion of the antioxidant agent, it is crucial to evaluate its release kinetics from the polymer matrix. The Korsmeyer–Peppas'model may be used to describe the Fickian and non-Fickian release behavior. Furthermore, the overall process was described by Gallagher and Corrigan **[16]** through two phenomenological independent contributions: the initial fast release or ''burst'' phase, in which the active compound undergoes a rapid dissolution process at the solid–liquid surface. The second phase is controlled by the polymer degradation mechanism. Several approaches have been reported on active compound release allowing the prediction of the delivery system parameters **[17-20]**.

This research emphasizes the use of ferulic acid as an antioxidant agent in the chitosan active film formulation. The aims of this work were: (i) to study the changes undergone by the chitosan matrix functionalized with ferulic acid as an antioxidant. Thus, both microstructure and physical properties such as solubility, thermal stability, barrier and mechanical properties were evaluated; (ii) to analyze new insights into the interactions between ferulic acid molecules and chitosan polymer through FTIR spectra and their relationship with chitosan-based film properties; iii) to determine the amount of the bioactive compound released from the chitosan matrix to a liquid medium; iv) to know the transport mechanism of ferulic acid and to model the kinetics of this process mathematically.

Experimental

Materials

Commercial chitosan from crab shells with a minimum deacetylation degree of 75% was purchased from Sigma (St. Louis, MO, USA). Analytical grade acetic acid was purchased from Anedra (Buenos Aires, Argentina) and ferulic acid (analytical grade) from Sigma-Aldrich (St. Louis, MO, USA).

Film-forming solution preparation

Chitosan solution of 1.5% (w/w) was prepared by solubilization in 1.5% (v/v) acetic acid solution at 20°C under continuous agitation for 12 h approximately, followed by a vacuum filtration to eliminate insoluble materials. A screening of ferulic acid (FA) concentration was assayed in order to select the optimum. Different amounts of this active compound (25, 50, 75, 100 mg FA/ g chitosan) were incorporated to chitosan solutions.

Film preparation

Chitosan films (CH) with and without FA were prepared by casting 20 g of filmogenic solutions onto Petri dishes (9 cm diameter) and drying at 37 °C in an oven until reaching constant weight (approximately 36 h). The obtained films were removed from the dishes and stored for 48 h prior to the determinations of structural, physicochemical, barrier and mechanical properties. Films were conditioned in a controlled room at 20 °C and 65 % relative humidity (RH) before doing the analysis.

Contact angle measurement

To determine film hydrophilicity, the surface water contact angle was performed by using a goniometer Ramé-Hart Model 500 (Ramé-Hart Instrument Co., USA) at room temperature. A drop of Milli-Q water was placed on the film surface and the evolution of the droplet shape was recorded with a video camera. Image analysis software (DRO Pimage Advanced v2.2) was used to determine the contact angle. A minimum of seven measurements, taken at different positions on the film, were carried out. The contact angles were measured on both sides of the drop and averaged.

UV barrier properties

The absorption spectra of CH based films were recorded in the wavelength range 200–600 nm by using a UV-visible Spectrophotometer (Hitachi U 1900, Japan).

Moisture content

Film moisture contents were determined by measuring their weight loss, upon drying in an oven at 105±1ºC until reaching constant weight (dry sample weight). Samples were analyzed at least in triplicate and results were expressed as grams of water per 100 g of sample.

Water vapor permeability

Water vapor permeability (WVP) tests were conducted based on a modified ASTM **[21]** method E96 using a

specially designed permeation cell that was maintained at 20°C as described in previous work **[22]**. After steady-state conditions were reached, eight measurements were performed over 8 h. Each informed value corresponded at least to four determinations.

Tensile stress-strain

Quasi-static test in uniaxial condition assays were conducted in a dynamic-mechanical thermal equipment Q800 (TA Instruments, New Castle, USA) using a tension clamp. A preload force of 1 N and a constant force ramp rate of 0.3 N min^{-1} were applied to record the stress-strain curves until rupture from film sample strips or up to 18 N. Tests were carried out at 25ºC. In order to calculate the elastic modulus at large deformations (E_C), stress-strain curves were fitted to Eq. (1):

$$\sigma_V = E_C \, \varepsilon_V \, e^{-\varepsilon_V K} \qquad (1)$$

where, ε_v and σ_v are the true strain and the true stress, respectively, E_C is the elastic modulus; *K* is a constant and it is regarded as a fitting parameter. From Eq. (1), the relationship between the stress (σ_v) and the true strain (ε_v) corresponds to the elastic module **[23]**. Samples were analyzed at least in triplicate.

Structural studies through microscopic studies

The morphology of the films was examined by using a transmission electron microscope (JEM 1200EX II Jeol, Japan) equipped with a digital camera (ES500W Erlangshen CCD Gatan). Small pieces from the center of the films were prepared according to Denavi et al. **[24]**. Morphology was also studied by scanning electron microscope (SEM) with a FEI model Quanta 200 electron microscope (The Netherlands).

Modulated differential scanning calorimetry (MDSC)

Modulated differential scanning calorimetric studies were performed over a temperature range from -100 ºC to 350 ºC using a DSC model Q100 controlled by a TA 5000 module (TA Instruments, New Castle, Delaware, USA), with a quench-cooling accessory, under a N_2 atmosphere (20 ml min^{-1}) and modulated capability. A standard heating ramp of 10ºC min^{-1} with a modulation period of 40 sec and a modulation temperature amplitude of 0.5ºC were chosen. The samples were weighed and prepared as described in previous work **[25]**. The first scan was performed from -100ºC up to 200ºC to limit possible chitosan degradation. After the first scan was completed, the sample was cooled until -100°C and then a second scan was recorded. All results were the average of two replicates.

The total, reversing and non-reversing signals were determined. The analysis of the thermograms was performed by using the Universal Analysis V1.7F software (TA Instruments).

Thermogravimetric analysis (TGA)

The TGA analysis of chitosan matrix and their modifications were carried out by using a Shimadzu TGA-50 (Japan). Samples were heated from 25 to 600ºC at a heating rate of 10 ºC min^{-1} under nitrogen atmosphere. The results were plotted as both the percentage of the weight loss and the first derivative of the weight loss as a function of temperature. Instruments Thermal Solutions, TA60 version 2.11 Software package was used for analyzing the curves in order to obtain thermal parameters.

Dynamic mechanical analysis (DMA)

DMA assays were conducted in dynamic-mechanical thermal equipment previously described using a tension clamp with a liquid N_2 cooling system as described in a preliminary study **[26]**. Multi-frequency sweeps (5, 10 and 15Hz) at a fixed amplitude (7 μm) from -90 to 200ºC at 5ºC min^{-1} were carried out, with an isotherm of 15 min at -90ºC. Each informed value corresponded at least to two determinations.

X-ray diffraction

Chitosan films with and without ferulic acid were analyzed by X-ray diffraction in an X'Pert Pro P Analytical Model PW 3040/60 (Almelo, The Netherlands). The CuK_α radiation (1.542 Å), operated at room temperature, was generated at 40 kV and 30 mA, and the relative intensity was recorded in the scattering range of (2θ) 3–60º with step size 2θ=0.02°.

FT-IR spectroscopy

The Fourier transform infrared (FT-IR) spectra of the films were recorded in an IR spectrometer (Nicolet, iS10 Thermo Scientific, Madison, USA) in the wavenumber range 4000-400 cm^{-1} by accumulation of 64 scans at 4 cm^{-1} resolution. Data were analyzed by using the software Omnic 8 (Thermo Scientific, Madison, USA).

Diffusion experiments

A study of the release of ferulic acid from the chitosan films was carried out by determining the diffusion of the antioxidant active compound in a 50: 50 (v/v) water: ethanol mixture. Film pieces of 4 × 4 cm, were placed on a support and immersed in 200 mL of the solvent, which was kept under agitation. Samples (0.2 mL) were taken from the liquid medium periodically (extract), and the concentrations of ferulic acid released from CH matrix to the medium were quantified by using the Folin-Ciocalteu method **[27]**.

The samples were allowed standing at room temperature for 2 min and then 0.2 mL of Folin-Ciocalteau reagent (1:1) were added. The mixture was stirred and, after a reaction time of 30 min., the absorbance was measured at 725 nm by using a spectrophotometer (Hitachi U 1900, Japan). The concentration of active compound in the extract was calculated from a standard curve. Samples were analyzed at least in triplicate.

The antioxidant activity of the active compound released was determined at a wavelength of 517 nm using the radical 2,2-diphenyl-1-picryl hydrazyl (DPPH •) (Sigma-Aldrich, USA). The Brand Williams et al. **[28]** protocol was followed with minor modifications, using the 50:50 water:ethanol mixture as the reaction medium. The

inhibition percentage of the radical was calculated according to López de Dicastillo et al. **[29]**.

Statistical analysis

Systat-software (SYSTAT, Inc., Evanston, IL, USA) version 10.0 was used for all statistical analysis. Analysis of variance (ANOVA), linear regressions and Fisher LSD mean comparison test were applied. The significance levels used was 0.05.

Results and discussion

Characterization of active films

CH matrix with different concentrations of FA showed a uniform appearance except those films with the addition of 100 mg FA/g CH. These last matrices were not considered for further tests since they had rough surfaces associated with the presence of an excessive amount of the active compound, which produced defects and lack of homogeneity in the material, which are shown in the photograph (**Fig. 1**). The observations by SEM also revealed matrix surface imperfections at microscopic level caused by the excess of FA in the films (**Fig. 1**).

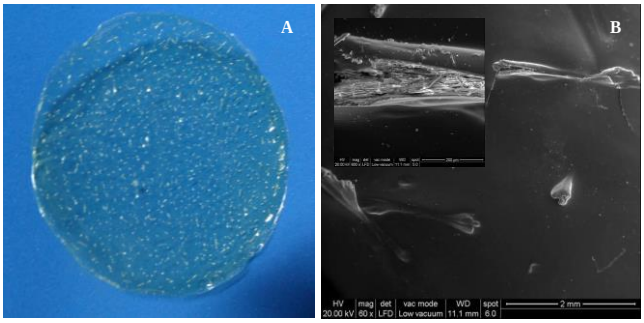

Fig. 1. Macroscopic observation (**a**) and SEM micrograph (**b**) obtained for chitosan films with 100 mg FA /g CH.

The effect produced by the addition of FA on the water vapor barrier properties is shown in **Fig. 2**. The addition of 50 mg FA/ g CH led to the lowest values of WVP, the differences being significant ($p<0.05$) compared with the control films.

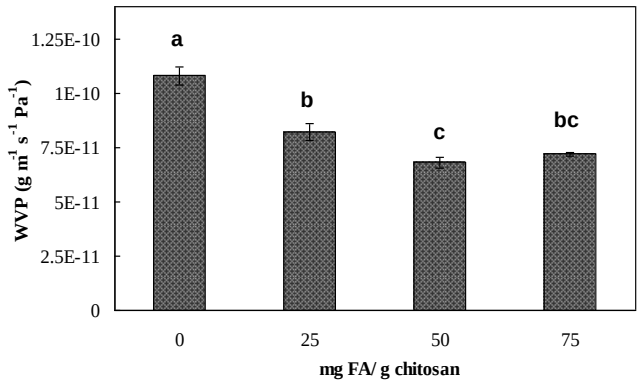

Fig. 2. Water vapor permeability (WVP) of CH films with different concentrations of ferulic acid (FA) incorporated to the matrix.

Cao et al. **[15]** stressed that the addition of ferulic acid to gelatin films did not significantly decrease the WVP. A similar trend was reported by Ou et al. **[4]** for soy protein isolates films.

While, Mathew and Abraham **[5]** informed a diminution around 18.4% in the WVP values for a concentration of 75 mg FA/ g polymer. They attributed these results to the arrangement of the polymers inside the film matrix which considerably affects the water vapor transfer property. Furthermore, Cao et al. **[15]** observed a slight influence of the addition of FA on the WVP values of gelatin films using concentrations in the range 0-40 mg FA/g polymer.

In parallel, mechanical tests were conducted on CH films with different concentrations of FA by using a DMA under uniaxial tension (**Fig. 3**). The addition of 50 mg FA/ g CH increased the tensile strength values around 14% over the CH films, while the elongation values showed a slight decrease. The most plausible explanation is the formation of a stable network promoted by the interactions caused by the incorporation of ferulic acid. Similar results were reported by Cao et al. **[15]** and Ou et al. **[4]**, who evaluating matrices based on soy proteins and gelatin attributed these results to a possible crosslinking effect of ferulic acid.

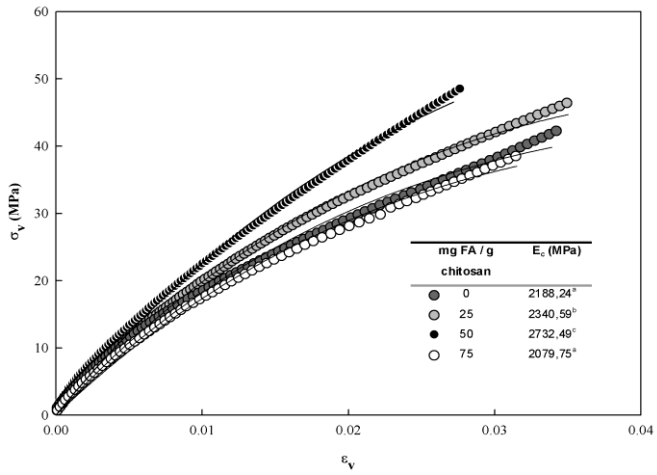

Fig. 3. Tensile stress-strain behavior of chitosan films with different concentrations of FA/ g CH. Continuous lines indicate data fitted by Eq. 1.

In all cases, the model used to estimate the elastic modulus fitted the experimental data satisfactorily ($r^2 \geq 0.99$). The elastic moduli (E_c) were determined from Eq. 1 as previously described. As it can be observed in Table insert in **Fig. 3**, the higher the FA concentration, the higher the E_c values, showing significant differences with those values obtained for chitosan film ($p<0.05$), except for the concentration of 75 mg FA/ g CH. In this last case, generated matrices exhibited weaker mechanical responses. According to Mathew and Abraham **[5]** the reduced resistance of the material with concentrations higher than 50 mg FA/ g CH could be attributed to an excess of the bioactive compound. The greater active compound/ polymer ratio should contribute to weak chemical bonds due to the large amount of free hydroxyl groups, which may promote interactions with similar hydroxyl groups and hence reduce the attraction forces with chitosan molecules. Based on the obtained results from the assessment of mechanical properties and water vapor barrier, the selected

ferulic acid concentration for further studies was 50 mg FA/ g CH.

Physicochemical properties

Solubility assays at 20 °C indicated that the addition of the antioxidant did not affect the CH film solubility, which turned out to be approximately 10% (**Table 1**). The contact angle measurement allows studying the surface hydrophilicity degree of the material which affects the adhesion properties as well as the kinetic release of the active compound molecules embedded in a matrix. **Table 1** shows the measure of contact angles of water drops deposited on chitosan films with and without the addition of FA. Ferulic acid significantly increased ($p<0.05$) the hydrophilicity of the CH films.

Table 1. Physicochemical properties of chitosan film control and matrices with 50 mg ferulic acid /g chitosan.

Film composition	Moisture content (g_{water}/ 100 $g_{dry\ film}$)	Solubility at 20°C** (%)	Contact angle (°)
CH	20.67 (0.80)[a]	10.57 (0.95)[a]	88.4 (5.1)[a]
CHFA	16.97 (0.31)[b]	9.85 (0.99)[a]	76.2 (3.5)[b]

* Different letters in the same column indicate significant differences ($P<0.05$) between samples.
** Solubility determined after immersion time of 60 min.

It is noteworthy that CH films have UV light barrier properties **[30]** and that FA has a UV absorption peak at 322 nm **[15, 31]**. The addition of FA to the matrix led to a shift of the maximum with the observation of the absorption peak between 308 and 342 nm and the improvement of the UV barrier regarding CH films. Therefore, chitosan active films can be used as an excellent barrier to prevent UV light-induced lipid oxidation when applied to food systems **[32]**.

Structural, thermal and spectroscopic properties

Fig. 4a shows TGA curves obtained for CH and CHFA films. The observed first stage between 25 and 160 °C was related to the loss of low molecular weight components bounded to water and adsorbed on the structure **[33]**. These values can be correlated to the film moisture content (**Table 1**). The addition of FA diminished the moisture content significantly ($p<0.05$).

The analysis of the samples by DSC showed an endothermic event at around 125°C (T_p) associated to the evaporation of the nonfreezable water present in the chitosan matrix with and without ferulic acid (**Fig. 4b**).

The differences found out between the enthalpy values of the samples coincided with the above findings regarding the moisture content of the materials (Table inserted in **Fig. 4b**). According to Neto et al. **[34]** polysaccharides usually have a strong affinity for water resulting in macromolecules with rather disordered structures. Therefore, variations in the enthalpy values evidenced by DSC and the position of the peaks related to water loss may reflect the molecular and physical changes caused by the interaction of the FA chains with chitosan polymer.

A detailed examination of **Fig. 4a** shows the differences in the percentage of mass loss and the onset temperature of the peak, indicating that these systems differ in their water holding capacity as well as in the strength of the interaction water-polymer-active compound.

From TGA curves was possible to determine the degradation temperature of the materials, which were correlated with the results obtained by MDSC. The analysis of the data derived by TGA indicated no significant difference ($p>0.05$) in the decomposition temperature of the samples, since the peaks were observed at 284 and 285°C for CH and CHFA films, respectively (**Fig. 4a**). Similar results were obtained by MDSC for the degradation temperature values (T_d). These findings showed that the addition of the active component did not affect the thermal stability of the polymer matrix. However, the weight loss involved in the stage between 200 and 400°C was minor for CHFA compared to CH films, obtaining values of 39.0 and 43.4 %, respectively.

In order to complement the study of thermal properties of the material, tests were also performed in DMA equipment. **Fig. 4c** depicts the dynamic mechanical profile of the samples; two relaxations, β and α, was found with increasing temperature. In the case of control CH film, T_β relaxation was located around -10ºC. Neto et al. **[34]** and Mucha and Pawlak **[35]** reported that chitosan films exhibited an event associated to β relaxation at around -20 ºC to -10 ºC, characteristic of the local motion of side groups in chitosan polysaccharide. On the other hand, the spectrum of CHFA revealed that the addition of the active compound caused the attenuation of this relaxation. The second peak in tan δ curves corresponded to α relaxation associated to the glass transition temperature (T_g). A sharp fall in the storage modulus values (E') was accompanied by an increase of tan δ values, locating the glass transition temperature at 100 ºC and 125°C for CH and CHFA films, respectively. This fact could be explained by considering the lower moisture content of the CHFA films together with chitosan-ferulic acid interactions, owing to their functional groups (hydroxyl and carboxyl groups).

These findings were also confirmed by MDSC assays. From the reversible heat flow signal of CHFA film, the glass transition temperature situated at 124.3°C was visualized, which is in agreement with the value obtained by DMA analysis. López de Dicastillo et al. **[29]** working with ethylene vinyl alcohol (EVOH) films with the addition of green tea extract as active compound, reported an increase in T_g values, indicating an increase in the stiffness with respect to EVOH control film. The authors attributed this effect to the interactions between the substituted OH of the polymer and the active agent, resulting in an increase in the force between chains and cohesive energy.

Microstructural analysis

Fig. 5 shows the sample micrographs by TEM. CH control films were homogeneous, with good structural integrity (**Fig. 5a**). The addition of FA led to the preferential orientation of the polymer matrix. The formation of an irregular structure with areas or domains distributed according to a certain alignment was possibly associated with the type of interactions established between the active compound and chitosan matrix.

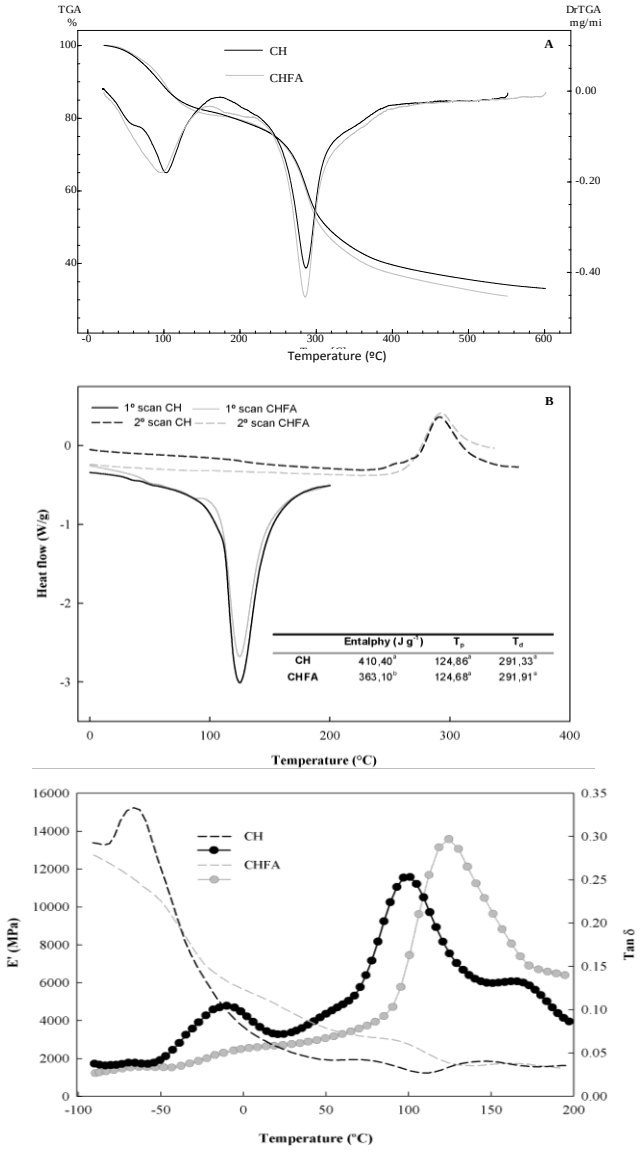

Fig. 4. Thermal properties: (**a**) TGA curves and their first derivatives obtained for CH and CHFA films; (**b**) MDSC thermograms showing 1º and 2º scan and (**c**) DMA spectrum showing the storage modulus E' (----) and tan δ(-●-) of CH and CHFA films. FA concentration: 50 mg/ g CH.

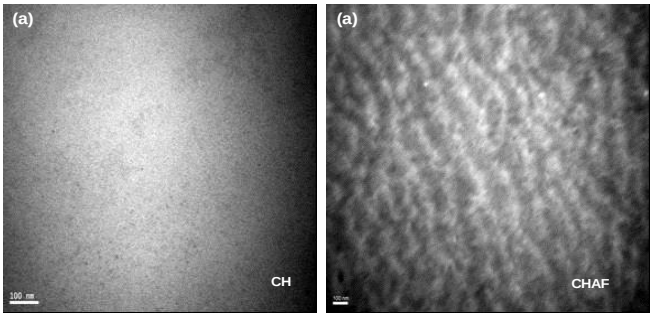

Fig. 5 TEM micrographs of: (**a**) chitosan films (CH) and (**b**) chitosan matrix with 50 mg ferulic acid/g chitosan (CHFA). Magnification: 100 nm between marks.

The X-ray diffractograms of control CH films exhibited three diffuse peaks located at 2θ = 11.4, 18.7 and 22.4° (**Fig. 6**). The addition of FA to the chitosan matrix intensified the characteristic peaks in control films. This fact could be explained by considering the rearrangement of the molecules involved in the interaction between the polymer and the active compound. The FA may induce inter and intramolecular hydrogen bridge leading to the formation of a structure with a different order as shown through TEM (**Fig. 5b**). The changes suffered by the material with the addition of FA, which could be observed by TEM had a correlation with the differences found in both barrier and mechanical properties. Moreover, Mathew and Abraham **[36]** and Yu et al. **[37]**, analyzing the diffraction patterns of ferulic acid, observed a behavior characteristic of a crystalline substance.

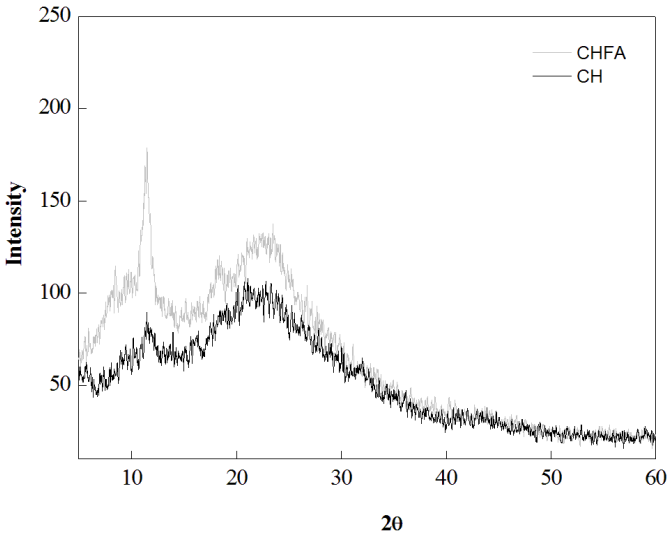

Fig. 6 X-ray diffractograms of chitosan films (CH) and chitosan films with ferulic acid (CHFA). FA concentration: 50 mg/ g CH.

The obtained results were supported by studies carried out through FTIR (**Fig. 7**). The FTIR spectrum of chitosan films showed characteristic peaks of amide I at 1650 cm^{-1} (C=O stretching), amide II at 1558 cm^{-1} (N-H in plane deformation coupled with C-N stretching) and amide III (C-N stretching coupled with NH in plane deformation) at 1314 cm^{-1} **[38]**.

Fig. 7 shows the absorption spectra of CH and CHFA, exhibiting a broad band in the region between 3600 and 3000 cm^{-1} with a maximum at 3460 cm^{-1} due to overlap stretching of OH and NH groups which would support the differences found in the moisture content of films. The other peaks at 2873 cm^{-1} and 1375 cm^{-1} were assigned to CH stretching and CH_3 symmetric deformation, respectively. The broad peak at 1076 cm^{-1} indicates CH stretching in chitosan.

FTIR studies revealed the changes caused by the incorporation of FA in the chitosan matrix. For clarity in identifying these modifications the region between 1700-1200 cm^{-1} was amplified (**Fig. 7a**) In addition, 2nd derivative was calculated as as shown in **Fig. 7b** and **7C**. The addition of FA produced shifts of some characteristic peaks of control films, since this active agent may interact with the matrix, mainly by hydrogen bonds through -OH and C=O groups. When compared with CH films, the CHFA matrix presented an absorption decrease at 1320, 1380 cm^{-1} (attributed to the NH-bending of the glucosamine unit) and 1420 cm^{-1} (the symmetric $-NH_3^+$

bending region) **[39]**. The decrease of these bands, associated with protonated glucosamine residues of chitosan, would be consistent with an interaction between FA and chitosan amino groups.

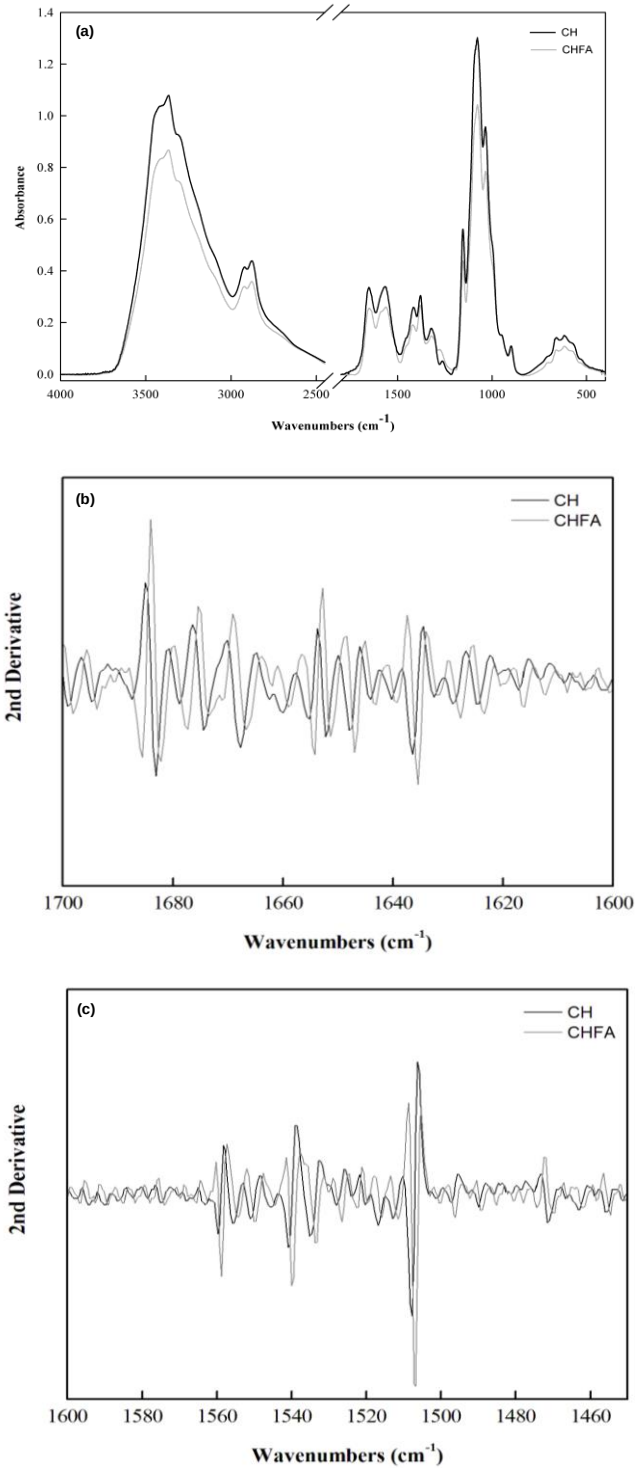

Fig. 7. (a) FTIR spectra of CH and CHFA films in the 3700-400 cm^{-1} wavenumber region. Insert shows the enlarged region 1700-1200 cm^{-1}; second derivatives of FTIR spectra of CHF and CHFA films in (b) 1700-1600 cm^{-1} and (c)1600-1450 cm^{-1} wavenumber region. FA concentration: 50 mg/ g CH.

The FTIR spectrum of CHFA films showed the appearance of two shoulders located at 1590 and 1516 cm^{-1} which are due to the vibrations of aromatic skeleton C=C of ferulic acid **[31, 37]**. Characteristic peak of C–O–C at 1280~1230 cm^{-1} was reduced owing to the addition of the acid. In accordance with Wang et al. **[40]** these results could indicate that polymer–active compound interaction plays a significant role on the ferulic acid release of the examined delivery system.

Release models

Upon immersion in the liquid medium (water: ethanol), the solvent penetrated into the chitosan matrix and dissolved the bioactive compound, which was then leached out of the film. Although the matrix underwent a dramatic swelling, releasing the bioactive compound due to absorption of large amounts of liquid, it was able to maintain its structural integrity behaving like a hydrogel. Korsmeyer et al. **[41]** proposed a simple semiempirical equation which can be used to analyze data concerning the controlled release of water soluble drugs from polymers. The antioxidant release mechanism was determined by fitting the first 60% of the release profile as suggested by Korsmeyer et al. **[41]** since in this region the model is valid. The experimental data was fitted with the following equation:

$$\frac{M_t}{M_\infty} = F_T = k\, t^n \qquad \text{Eq. (2)}$$

where, M_t/M_∞ is the release fraction of antimicrobial compound at time *t*, *k* is a constant and *n* (the release exponent) is a parameter indicative of the mechanism of transference of the active agent.

The obtained results revealed that complete transference from CHFA films was achieved when the films were immersed into the liquid medium. The mathematical model (Eq.2) adequately predicted the mechanism of ferulic acid release from the film. The diffusion exponent obtained from the Eq. 2 was $n = 0.5$, confirming that the release of the antioxidant active compound would be mainly governed by diffusion. However, taking the matrix swelling into account, these results suggested that the release of the antioxidant agent was controlled by two parallel mechanisms, one Fickian-type and the other associated to the high degree of the matrix swelling. Rivero et al. **[16]**, studying CH films with propionic acid, found that the delivery of the active compound was not only controlled by a swelling process but also the diffusion contributed to the mass transfer from films.

Experimental data were fitted with the model of Gallagher and Corrigan **[17]** that estimates the total fraction of compound released at time t (F_{TOT}), which is given by the sum of additive transferred by surface diffusion and that released by degradation of the polymer matrix as follows

$$F_{TOT} = F_B(1 - e^{-K_B t}) + (1 - F_B)\left(\frac{e^{kt - kt_{MAX}}}{1 + e^{kt - kt_{MAX}}}\right) \qquad (3)$$

where, k_B is the burst rate constant, F_B is the total burst fraction at infinite time, t_{MAX} is the time for maximum rate and *k* the rate constant of the polymer degradation release phase. The kinetic profile of this model is described as an initial "burst effect" of an active compound non-bound to the matrix, followed by a slow release determined by the matrix swelling **[16, 17, 20]**. **Fig. 8** shows the release

profile of ferulic acid in the liquid medium and the model approximated the experimental points satisfactorily (r^2 = 0.99) as it can be observed.

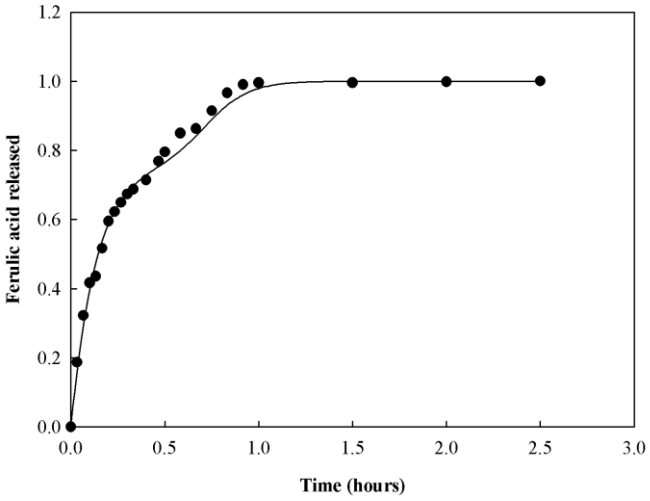

Fig. 8 Release profile of ferulic acid from chitosan films (functionalized with 50 mg FA/ g CH) to liquid medium (water:alcohol) (●). Experimental data (----) were fitted by Eq. 2.

The parameters of the first (K_B = 7.53 h^{-1}) and the second release stage (k = 8.65 h^{-1}) were estimated from the model (Eq. 3). The obtained release profile confirmed that the process was driving by diffusion at the initial phase release (burst stage) and a faster second one, in which the matrix swelling became the main mechanism of the agent delivery. The time required to reach the second stage as well as that necessary to achieve the complete release was t_{MAX} = 0.72 h (**Fig. 8**). Similar results were reported by Aldana et al. **[20]** working on drug release from chitosan based films.

The fast FA displacement and release from the CH films could be attributed to favorable interactions between solvent and network. According to Aldana et al. **[19]**, CH film has enough free amino and hydroxyl groups to form a significant amount of hydrogen bond with the solvent and allow the matrix swelling. These factors contribute to the migration of the bioactive compound from the network. Once the mechanisms and release profiles of the compound active was established, the antioxidant capacity of the FA released was determined by the DPPH method. The inhibition percentage of ferulic acid released was estimated by considering the asymptotic values of the delivery profiles, being 18% for chitosan films containing FA as an antioxidant agent.

Conclusion

In summary, examining the obtained results from the analysis of interactions between chitosan and ferulic acid, the latter demonstrated to be incorporated in the polymer matrix. The addition of the active component allowed tailoring the functionality of the chitosan films and modifying microstructure and physical film properties such as solubility, thermal stability, barrier and mechanical properties. Consequently, it caused a decrease in both the moisture content and permeability to water vapor, an increase in resistance and a change at the structural level evidenced by TEM. The developed matrix is capable of releasing ferulic acid, exhibiting antioxidant properties and expanding the spectra for new applications of active materials. The antioxidant and UV-barrier properties induced by the addition of ferulic acid turned the chitosan films into a potentially active material to be applied on high-fat foods, however further studies are necessary.

Reference

1. Gómez-Estaca, J.; López-de-Dicastillo, C.; Hernández-Munoz, P.; Catalá, R.; Gavara, R. *Trends Food Sci.Tech.* **2014**, *35*, 42. **DOI:** 10.1016/j.tifs.2013.10.008
2. Carrizo, D.; Gullo, G.; Bosetti, O.; Nerína, C. *Food Addit. Contam. Part A.* **2014**. **DOI:** 10.1080/19440049.2013.869361
3. Shukla. S.K.; Mishra, A.K.; Arotiba, O.A.; Mamba, B.B. *Int. J. Biol. Macromol.* **2013**, *59*, 46. **DOI:** 10.1016/j.ijbiomac.2013.04.043
4. Ou, S.; Wang, Y.; Tang, S.; Huang, C.; Jackson, M.G. *J. Food Eng.* **2005**, *70*, 205. **DOI:** 10.1016/j.jfoodeng.2004.09.025. ISSN: 0260-8774.
5. Mathew, S.; Abraham, T.E. *Food Hydrocolloid.* **2008**, *22*, 826. **DOI:** 10.1016/j.foodhyd.2007.03.012.
6. Itagaki, S.; Kurokawa, T.; Nakata, C.; Saito, Y.; Oikawa, S.; Kobayashi, M.; Hirano, T.; Iseki, K. *Food Chem.* **2009**, *114*, 466. **DOI:** 10.1016/j.foodchem.2008.09.073.
7. Ou, S.; Kwok, K-C. *J. Sci. Food Agric.* **2004**, *84*, 1261. **DOI:** 10.1002/jsfa.1873.
8. Srinivasan, M.; Sudheer, A.R.; Menon, V.P. *J Clin Biochem Nutr.* **2007**, *40*, 92. **DOI:** 10.3164/jcbn.40.92.
9. Woranucha, S.; Yoksan R. *Carbohyd. Polym.* **2013**, *96*, 495. **DOI:** 10.1016/j.carbpol.2013.04.006.
10. Liu, J.; Wen, X-y; Lu, J-f; Kan, J.; Jin, C-h. *Int. J. Biol. Macromol.* **2014**, *65*, 97. **DOI:** 10.1016/j.ijbiomac.2014.01.021.
11. Prashanth, K.V.H.; Tharanathan, R.N. *Trends Food Sci.Tech.* **2007**, *18*, 117. **DOI:** 10.1016/j.tifs.2006.10.022
12. Rinaudo, M. *Polym.Sci.* **2006**. *31*, 603. **DOI:** 10.1016/j.progpolymsci.2006.06.001
13. Wessling, C.; Nielsen, T.; Leufven, A.; Jagerstad, M. *J. Sci. Food Agric.* **1999**, *79*, 1635. **DOI:** 10.1002/(SICI)1097-0010(199909)79:12<1635::AID-JSFA413>3.0.CO;2-#.
14. Wessling, C.; Nielsen, T.; Giacin, J.R. *J. Sci. Food Agric.* **2001**, *81*, 94. **DOI:** 10.1002/1097-0010(20010115)81:2<194::AID-JSFA801>3.0.CO;2-R.
15. Cao, N.; Fu, Y.; He, *J. Food Hydrocolloid.* **2007**, *21*, 575. **DOI:** 10.1016/j.foodhyd.2006.09.001.
16. Gallagher, K.M.; Corrigan, O.I. *J. Control. Release.* **2000**, *69*, 261. **DOI:** 10.1016/S0168-3659(00)00305-9.
17. Rivero, S.; García, M.A.; Pinotti, A. *J. Food Eng.* **2013**, *116*, 524. **DOI:** 10.1016/j.jfoodeng.2012.12.025.
18. Siepmann, J.; Peppas, N.A. *Adv. Drug Deliv. Rev.* **2001**, *48*, 139. **DOI:** 10.1016/S0169-409X(01)00111-9.
19. Flores, S.; Conte, A.; Campos, C.; Gerschenson, L.; Del Nobile, M. *J. Food Eng.* **2007**, *81*, 580. **DOI:** 10.1016/j.jfoodeng.2006.12.010.
20. Aldana, A.A.; González, A.; Strumia, M.C.; Martinelli, M. *Mater. Chem. Phys.* **2012**, *134*, 317. **DOI:** 10.1016/j.matchemphys.2012.02.071.
21. ASTM. (1995). Standard test methods for water vapor transmission of material, E96-95. *Annual book of ASTM*: Philadelphia, PA: American Society for Testing and Materials.
22. Rivero, S.; García, M.A.; Pinotti, A. *Carbohyd. Polym.* **2010**, *82*, 270. **DOI:** 10.1016/j.carbpol.2010.04.048.
23. León, P.G.; Chillo, S.; Conte, A.; Gerschenson, L.N.; Del Nobile, M.A.; Rojas, A.M. *Food Hydrocolloid.* **2009**, *23*, 1660. **DOI:** 10.1016/j.foodhyd.2008.12.008.
24. Denavi, G.; Tapia-Blácido, D.R.; Añón, M.C.; Sobral, P.J.; Mauri; A.N.; Menegalli, F.C. *J. Food Eng.* **2009**, *90*, 341.

DOI: 10.1016/j.jfoodeng.2008.07.001.
25. Rivero, S.; García, M.A.; Pinotti, A. Innov. *Food Sci. Emerg. Technol.* **2010**, *11*, 369.
DOI: 10.1016/j.ifset.2009.07.005.
26. Rivero, S.; García, M.A.; Pinotti, A. J. Agric. Food Chem. **2012**, *60*, 492.
DOI: 10.1021/jf204077k.
27. Schlesier, K.; Harwat, M.; Böhm, V.; Bitsch, R. *Free Radical Research.* **2002**, *36*, 177.
DOI: 10.1080/10715760290006411.
28. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Levesm. Wiss. U. *Technol.* **1995**, *28*, 25.
DOI: 0023-6438/95/010025.
29. López-de-Dicastillo, C.; Nerín, C.; Alfaro, P.; Catalá, R.; Gavara, R.; Hernández-Muñoz, P. *J. Agric. Food Chem.* **2011**, *59*, 7832.
DOI: 10.1021/jf200749f.
30. Leceta, I.; Guerrero, P.; Ibarburu, I.; Dueñas, M.T.; de la Caba, K. *J. Food Eng.* **2013**, *116*, 889.
DOI: 10.1016/j.jfoodeng.2013.01.022.
31. Sebastian, S.; Sundaraganesan, N.; Manoharan, S. *Spectrochim. Acta Mol. Biomol. Spectros.* **2009**, *74*, 312.
DOI: 10.1016/j.saa.2009.06.011.
32. Martins, J.T.; Cerqueira, M.A.; Vicente, A.A. *Food Hydrocolloid.* **2012**, *27*, 220.
DOI: 10.1016/j.foodhyd.2011.06.011.
33. Martucci, J.F.; Vázquez, A.; Ruseckaite, R.A. *J. Thermal. Anal. Calorim.* **2007**, *89*, 117.
DOI: 10.1007/s10973-006-7454-0.
34. Neto, C.G.T.; Giacometti, J.A.; Job, A.E.; Ferreira, F.C.; Fonseca, J.L.C.; Pereira, M.R. *Carbohyd. Polym.* **2005**, *62*, 97.
DOI: 10.1016/j.carbpol.2005.02.022.
35. Mucha, M.; Pawlak, A. *Thermochim. Acta.* **2005**, *427*, 69.
DOI: 10.1016/j.tca.2004.08.014.
36. Mathew, S.; Abraham, T.E. *Food Chem.* **2007**, *105*, 579.
DOI: 10.1016/j.foodchem.2007.04.032.
37. Yu, D-G.; Yang, J-M.; Branford-White, C.; Lu, P.; Zhang, L.; Zhu, L-M. *Int. J. Pharm.* **2010**, *400*, 158.
DOI: 10.1016/j.ijpharm.2010.08.010.
38. Amaral, I.F.; Granja, P.L.; Barbosa, M.A. *J. Biomater. Sci. Polym. Edn.* **2005**, *16*, 1575..
39. Aljawisha, A.; Chevalot, I.; Piffauta, B.; Rondeau-Mouroc, C.; Girardina, M.; Jasniewskia, J.; Schera, J.; Muniglia, L. *Carbohyd. Polym.* **2012**, *87*, 537.
DOI: 10.1016/j.carbpol.2011.08.016.
40. Wang, S.; Gao, Z.; Chen, X.; Lian, X.; Zhu, H.; Zheng, J.; Sun, L. *Biomed. Mater.* **2008**, *3*, 1.
DOI: 10.1088/1748-6041/3/3/034122.
41. Korsmeyer, R.W.; Gurny, R.; Doelker, E.M.; Buri, P.; Peppas, N.A. *Int. J. Pharm.* **1983**, *15*, 25.
DOI: 10.1016/0378-5173(83)90064-9.

