Carbohydrate Polymers 110 (2014) 374–381

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Active naringin-chitosan films: Impact of UV irradiation

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ARTICLE INFO

Article history:
Received 3 December 2013
Received in revised form 13 March 2014
Accepted 17 March 2014
Available online 2 April 2014

Keywords:
Citrus extract
Naringin
Chitosan
Films
Antimicrobial activity
UV irradiation

ABSTRACT

Bioactive citrus extract-chitosan films were prepared through solvent casting-evaporation method. The impact of near UV irradiation was studied to reach a better understanding of the film behavior. The antimicrobial activity of films against *Listeria innocua* was maintained after UV irradiation. To study the interaction between chitosan and citrus extract components, naringin (main component) was selected as the model compound. UV treatment caused modifications of the flavanone regardless of the solvent used for its dissolution, depending on the concentration of naringin in the film: the greater the concentration the lower the modification. DSC results suggested cross-links due to UV irradiation and interactions between naringin and chitosan. This was confirmed by a decrease in the naringin release from the irradiated samples. Naringin- and citrus extract-chitosan films showed an increased absorbance in the UV region compared to pure chitosan films, showing potentiality for decreasing the lipid oxidation induced by UV light in foodstuffs.

1. Introduction

Over the last decade new antimicrobial materials for food packaging have been a matter of great interest and continuous research in terms of food safety, food quality, and shelf life improvement (Campos, Gerschenson, & Flores, 2011; Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011). For the development of these materials, antimicrobial compounds are being studied for incorporation into polymer matrices.

The use of natural extracts from plants, herbs, spices and fruits and some of their components, have arisen as a promising alternative to synthetic preservatives (Burt, 2004; Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011), in order to fulfill consumers' demand for natural, safe, and high-quality food products. Citrus fruits extract, which are composed by extracts of different fruits such as grapefruit, sweet orange, bergamot and mandarin, has proven antimicrobial effectiveness against different food spoilage bacteria and pathogens (Corbo et al., 2008; Del Nobile et al., 2009). It is rich in flavonoids (Benavente-García, Castillo, Marin, Ortuño, & Del Río, 1997), which have shown antibacterial, antitoxin, antiviral and antifungal activities (Friedman, 2007). 7-*O*-Glycosylflavanones, such as naringin (Fig. 1), are the most abundant flavonoids in all the species in the genus *Citrus* (Benavente-García et al., 1997). Naringin may act as an antimutagenic agent (Ortuño et al., 2006) and exhibits antibacterial (Sharma, 2006) and antifungal activity (Ortuño et al., 2006). Rutin, quercetin and isoquercitrin flavonols (Fig. 1) have also been found in citrus extract (Mata Bilbao, Andrés-Lacueva, Jaúregui, & Lamuela-Raventós, 2007). Flavonoids and flavonoid-related compounds have been incorporated into films made of both synthetic and natural polymers (Helal, Tagliazucchi, Conte, & Desobry, 2012; López-de-Dicastillo, Alonso, Catalá, Gavara, & Hernández-Muñoz, 2010) or grafted on different biomacromolecules (Curcio et al., 2009; Sousa, Guebitz, & Kokol 2009) for obtaining materials with antioxidant or antimicrobial functionalities or even for cross-linking purposes (He et al., 2011; Yan, Li, Zhao, & Yi, 2011). Bio-based polymers are gaining much attention due to environmental problems and potential valorization of residues arising from food industry or agriculture and fishery. Chitosan, a biopolymer composed of *N*-acetyl-D-glucosamine and D-glucosamine units linked by β-(1→4) bonds, has been widely studied due to its good film forming properties and its broad spectrum of antimicrobial activity against bacteria, fungi, yeast and molds (Kong, Chen, Xing, & Park, 2010), connected to its unique polycationic nature. Moreover, its immunoadjuvant capacity and absence of toxicity toward

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http://dx.doi.org/10.1016/j.carbpol.2014.03.062
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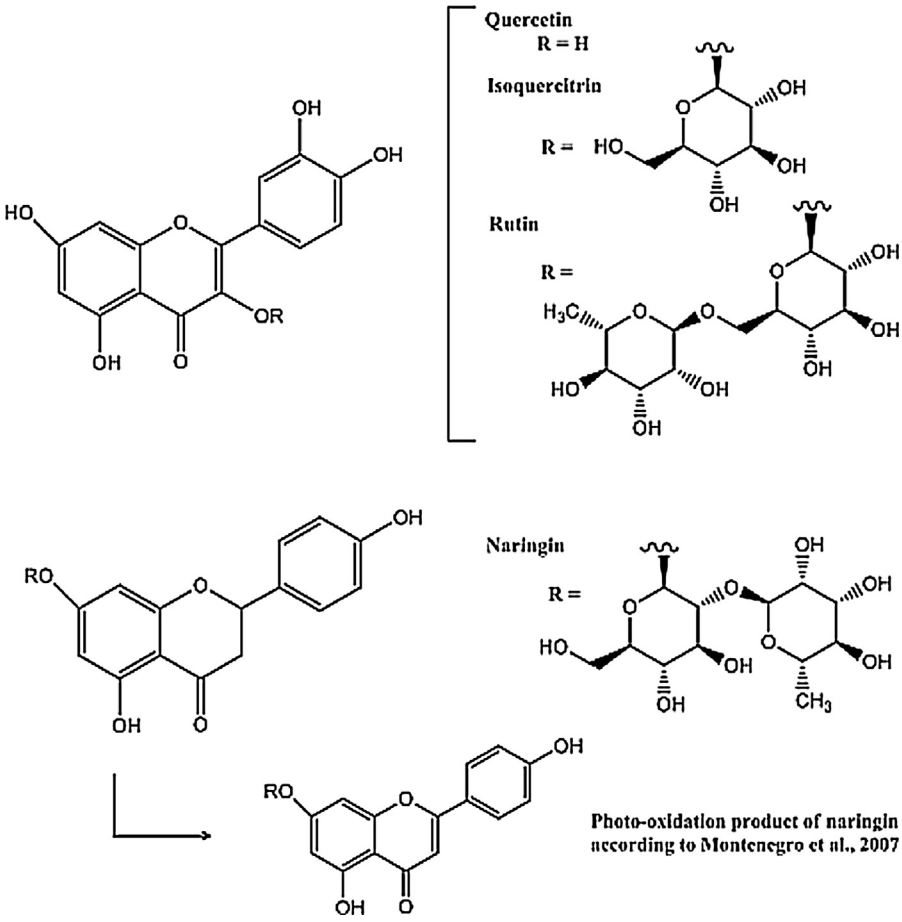

Fig. 1. Chemical structures of the main components of citrus extract and photooxidation product of naringin according to Montenegro et al. (2007).

mammalian cells makes it a potential biocide for food preservation and for other applications such as biomedical, agricultural, biotechnological, and pharmaceutical ones (Muzzarelli et al., 1990; Muzzarelli, 2009, 2010).

Disadvantages, mainly water sensitivity and lower mechanical properties, presented by biopolymers as compared to synthetic polymers can be overcome or improved by the formation of intermolecular associations by enzymatic, physical, and chemical cross-linking techniques. Cross-linking can be used to enhance mechanical strength and chemical stability, control aqueous permeability and solubility, reduce aqueous swelling features of chitosan-based materials (Moura, Figueiredo, & Gil, 2007), and also to decrease the release of antimicrobial compounds incorporated into these matrices. Among the physical cross-linking methods, UV irradiation provides a simple and environmental friendly method to change the physical, chemical, and/or biological characteristics of a product (Ummi-Shafiqah, Fazilah, Karim, Kaur, & Yusup, 2012). This technique has proven its effectiveness for the modification of chitosan-based films (Sionkowska et al., 2006).

In sunlight, mainly the ultraviolet radiation determines the useful lifetime of many materials (Sionkowska, 2006). UV irradiation (250 to about 340 nm) can create changes in the average molecular weight of chitosan (Andrady, Hamid, Hu, & Torikai, 1998) and degradation, oxidation, or photochemical transformations in flavonoids (Montenegro, Nazareno, & Borsarelli, 2007; Sisa, Bonnet, Ferreira, & Van der Westhuizen, 2010). Thus, the study of these materials under UV irradiation is necessary for a better understanding of their behavior and to try to foresee the consequences of this exposition over their performance.

In this paper, the antimicrobial activity of chitosan films associated to citrus extract was evaluated before and after UV irradiation. The interactions in flavonoid-chitosan films treated with UV irradiation and the modifications of their bioactive properties were studied using naringin as model compound.

2. Experimental

2.1. Materials and microorganisms

Citrus extract was obtained from Agrobiosys (Barcelona, Spain). Naringin (7-rhamnoglucosyl-4′,5-dihydroxyflavanone) was purchased from Acros Organics (97%, Fisher Scientific, Illkirch, France). Chitosan (Low Mw, viscosity 20,000 cps) and 2,2-diphenyl-1-picryl hydrazyl (DPPH) were purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). All the reagents and solvents were analytical grade, and the water used throughout was distilled. Antimicrobial properties were determined against *Listeria innocua* 430 (ENSCBP collection, University of Bordeaux 1, France). This strain was grown in tryptose broth (Difco 262200, Fisher Scientific Bioblock, Illkirch, France) at 37 °C for 18 h.

2.2. Film formation and irradiation

Active films were prepared by dispersing chitosan in a 1% (w/w) aqueous glacial acetic acid solution to obtain a 1% (w/v) film forming solution (FFS). The solutions were slowly stirred for 2 h for chitosan complete dissolution and bubble elimination.

Citrus extract-chitosan (CE-chitosan) films: Citrus extract was directly added into chitosan FFS to obtain a final concentration of 0.5% (v/v).

Naringin-chitosan (N-chitosan) films: Naringin stock solution (3% w/v) was prepared by dissolving naringin in 1 M NaOH solution or in absolute ethanol, and then was slowly added with continuous stirring into chitosan FFS to obtain final concentrations of 0.05, 0.1, 0.2, 0.3, 0.5 and 1.0% (w/v).

Control FFS were prepared in the same way as active films but with the addition of 1 M NaOH solution or absolute ethanol instead of naringin solution, and distilled water instead of citrus extract. Both control and active viscous solutions were stirred and filtered through a filter cloth to remove any impurity or undissolved matter. The pH of the filtered solutions for chitosan and N-chitosan was below 6.0 for every formulation. Films were obtained by casting the solutions onto polystyrene plates so as to obtain 0.32 g cm^{-2}. CE-chitosan and its corresponding control film (without active extract) were dried in an oven at 40 °C for 20–24 h, whereas the films containing naringin and their respective controls were dried at 80 °C for 3 h to avoid naringin crystallization due to its low solubility in aqueous media. Film thicknesses, measured by a micrometer (Mitutoyo Manufacturing, Japan) at 10 random positions around three replicates of each film, were found to be 0.032 ± 0.001 mm and 0.044 ± 0.003 mm for chitosan and N-chitosan films respectively. Films were then irradiated for 6 h with 4 black light UV lamps (Mazdafluor TFWN 18) emitting mainly at 350 nm. The distance between the lamps and the films was 15 cm and the light intensity delivered at this distance was homogeneous and estimated to be in the order of 0.25 mW cm^{-2} using Aberchrome 540 as an actinometer (Gardrat et al., 2004). The temperature in the irradiation setup was maintained at 25 °C by means of a fan.

2.3. Antimicrobial activity assays

The antibacterial properties of chitosan-based films were determined using an agar plate method performed by a disk diffusion method. To do this, 20 mL of culture medium prepared by mixing tryptose broth (Difco 262200, Fisher Scientific Bioblock, Illkirch, France.) with 15% (w/w) agar (Difco 215530, Fisher Scientific Bioblock, Illkirch, France) was poured into each Petri dish. Then, 100 μL of an 18 h microbial culture, which was previously diluted to 10^3, was gently spread on the surface of the agar plate. After a few minutes, 6 mm-diameter film disks were placed on the surface of inoculated agar plates. Thereafter, plates were incubated at 37 °C for 24 h. The inhibition diameters, indicated by clear zones surrounding the disks, were then measured and recorded. Each test was performed in triplicate.

2.4. UV–vis spectroscopy

The photochemical changes arisen from the UV irradiation of the films were analyzed by UV–visible spectrometry using a Perkin–Elmer Lambda 18 spectrometer (Perkin–Elmer, Courtaboeuf, France). The absorption UV–vis spectra of the films were recorded in the range between 200 and 700 nm. The absorption spectra of naringin at different concentrations of NaOH in distilled water (0.1, 1.0, 10, and 100 mM) were also recorded for studying the effect of pH on the absorption spectra and the structure of naringin.

2.5. Thermal characterization

Thermal properties of the films were determined using a DSC Q-2000 differential scanning calorimeter (TA Instruments-Waters LLC, New Castle, USA). Before the analysis, the films were conditioned for at least 5 days in a desiccator with silica gel. Samples (5–10 mg) were sealed in aluminum pans, using an empty pan as a reference. The temperature range used was 0 to 200 °C with a heating rate of 10 °C/min in an inert atmosphere (N_2 flow 50 mL/min). The glass transition temperature (T_g) and the melting (peak) temperature (T_m) were calculated using TA universal thermal analyzer software (Universal® Analysis 2000, version 4.5A, TA instruments, New Castle, USA) and were represented as the average of three replicates ± standard deviation (SD).

2.6. Naringin release profile from chitosan films and DPPH radical scavenging activity

A fatty food simulant was selected, ethanol 95% (v/v), as the target application of these films is fish preservation. The release of naringin from the films (control and UV irradiated) was carried out by determining the specific migration of the compound, from the chitosan films into the simulant by UV–vis spectroscopy (Perkin–Elmer Lambda 18 spectrometer, Perkin–Elmer, Courtaboeuf, France) at 331 nm ($\varepsilon_{naringin}$ = 3615 L mol^{-1} cm^{-1}). Migration tests were carried out at room temperature (21 °C) as described by López-de-Dicastillo et al. (2010) with slight modifications. The films used in this assay were the ones prepared with naringin at concentration of 1.0% (w/v) (dissolved in ethanol) in the FFS. Entire films of 53 cm^2 were totally immersed in 250 mL of the simulant (area-to-volume ratio around 2 dm^2/L) under magnetic stirring at 150 rpm in glass vessels covered with aluminum foil to protect naringin from light. At different periods of time an aliquot (3 mL) of each released solution was withdrawn and the absorption spectra of the solutions were recorded, being then returned into the release medium. Films were tested in triplicate and results were expressed as the mean concentration of naringin released into the simulant, determined by a calibration curve.

The reduction capability of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) was determined by its absorbance decrease at λ_{max} = 519 nm, as induced by the antioxidant. Radical scavenging activity was determined as described by Portes, Gardrat and Castellan (2007), with a slight modification. A stock solution (5.0 mg mL^{-1}) of naringin was made up in ethanol 95% (v/v) and then diluted to prepare the concentration series for naringin antioxidant capacity determination. One mL of DPPH solution (150 μM) in 95% ethanol was added to 1.0 mL of tested sample. The mixture was then vigorously mixed and allowed to stand at room temperature in the dark for 30 min. Inhibition of free radical DPPH in percent (I%) was calculated as: $I\% = [(A_{control} - A_{sample})/A_{control}] \times 100$; where $A_{control}$ is the absorbance of the control reaction containing all reagents except naringin, and A_{sample} is the absorbance of the test sample. All tests were carried out in triplicate and inhibition percentages were reported as mean ± SD. The inhibition percentages of the released naringin solution were determined when all the naringin was released into the solution, *i.e.*, after 24 h of immersion of the film into the simulant.

3. Results and discussion

3.1. CE-chitosan films

3.1.1. UV–vis characterization of CE-chitosan films

UV–vis spectra of chitosan and CE-chitosan (0.5% v/v) films are shown respectively in Figs. 2 and 3. The former revealed only a broad band at about 290–320 nm, in accordance with the literature (Andrady, Torikai, & Kobatake, 1996; Sionkowska et al., 2006) and the latter presented higher absorbances both in the UV (200–380 nm) and the visible (380–600 nm) ranges, suggesting that incorporation of citrus extract could potentially prevent the lipid oxidation induced by UV and visible light in a food system.

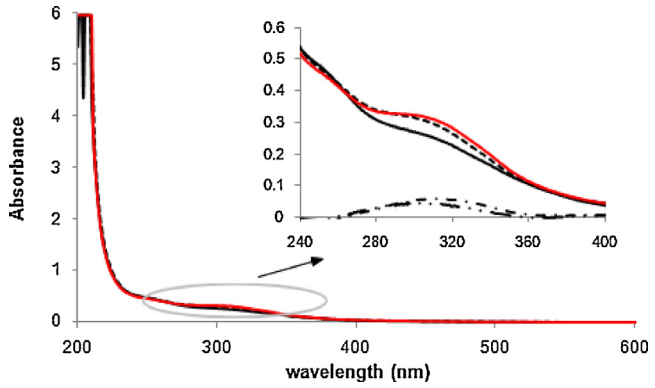

Fig. 2. UV–vis spectra of chitosan films: (—) control film; (– –) irradiated 6 h; (▬): irradiated 6 h then left in darkness for 24 h; (— · · —) absorption difference between films irradiated 6 h and control; (– · –): absorption difference between films irradiated 6 h then left in darkness for 24 h and control.

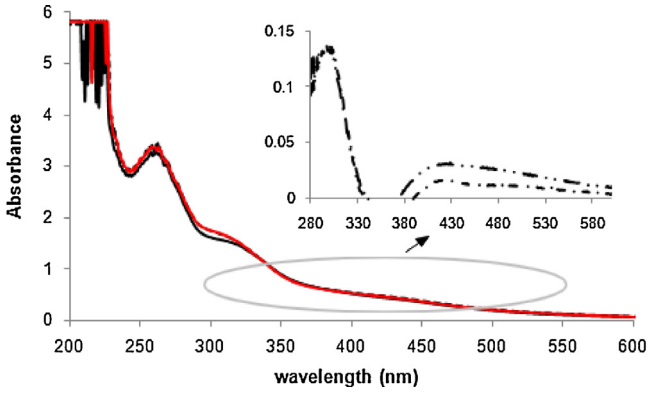

Fig. 3. UV–vis spectra of CE-chitosan based (1% w/v–0.5% v/v) films: (—) control; (– –) irradiated 6 h; (▬): irradiated 6 h then left in darkness for 24 h. Insert: (— · · —) absorption difference between films irradiated 6 h and control; (– · –) absorption difference between films irradiated 6 h then left in darkness for 24 h and control.

After irradiation both chitosan and CE-chitosan films showed higher absorbances. Irradiation conditions were chosen to simulate the effect of near UV natural light on the aging of natural materials (Sundaryono et al., 2003). Differences between spectra (irradiated *vs* non-irradiated) evidenced that, for irradiated chitosan films, a new band was formed, which was shifted to longer wavelengths after standing in the dark for 24 h. For CE-chitosan films, the same operation revealed also the formation of a new band (at higher wavelength than for chitosan films), but the latter almost did not change after leaving the film in the dark for 24 h. The behavior of chitosan films irradiated by UV light was in accordance with the literature (Andrady et al., 1996): a new band, likely due to the formation of carbonyl and amino chromophores after the breakdown of chitosan chains, appeared at 310 nm.

3.1.2. Antimicrobial activity of CE-chitosan films

Fig. 4 illustrates the antimicrobial activity of CE-chitosan (chitosan 1% w/v, citrus extract 0.5% v/v) and chitosan (1% w/v) films. CE-chitosan films showed inhibition zones against *L. innocua* (Fig. 4B), which were maintained after UV irradiation for 6 h (Fig. 4C). Citrus extract has already proven its antilisterial activity in a previous work (Iturriaga, Olabarrieta, & Martínez de Marañón, 2012). Thus, in our experimental conditions, UV irradiation seems not to degrade the active compounds of the citrus extract (flavonoids), which are known to protect plants against UV (Gould & Lister, 2006) and cells against oxidation (Montenegro et al., 2007).

Inhibition zones of around 25–26 mm, film diameter (6 mm) included, were obtained for both irradiated and non-irradiated CE-chitosan films. The appearance of a clear zone around film disks denoting bacterial growth inhibition is due to the diffusion of the antimicrobial compounds of citrus extract through the agar layer. Therefore, the antimicrobial activity obtained by diffusion of active compounds would be added to the non-migrating antimicrobial potency of chitosan (Fig. 4B *vs.* A), as already observed by Pranoto, Rakshit and Salokhe (2005) and described by Mayachiew, Devahastin, Mackey and Niranjan (2010) for galangan extract-chitosan films.

3.2. N-chitosan films

Among the flavonoids present in citrus extract, naringin proved to be the most soluble in water and ethanol. For a better understanding of the interactions between chitosan and citrus extract, this compound was selected and incorporated into chitosan films using two different solvents, aqueous NaOH (1 M) and absolute ethanol.

3.2.1. UV–visible absorption of naringin in alkaline solutions

The UV-visible spectra of naringin with various amounts of sodium hydroxide were presented in Fig. 5. In basic conditions, naringin exists both in flavanone (cyclized structure FL) and chalcone (open structure CH) forms in equilibrium. It has already been observed that in acid and neutral solutions of protic solvents, the flavanone form of naringin is largely stable (González, Nazareno, & Borsarelli, 2002), giving bands with absorption maxima at 285 and 330 nm. In this study, the same bands were obtained for pH = 9.4 (0.1 mM NaOH solution) and 10.6 (1.0 mM NaOH solution) (Fig. 5). When using higher concentrations of sodium hydroxide solutions (0.01 and 0.1 M) for the preparation of naringin solutions, instantaneous bathochromic shifts on the flavonoid spectra were observed; new bands at 245 and 365 nm appeared, due to deprotonation of the phenolic hydroxyl groups of flavanone structure to give flavanone in its anionic form (FL^{2-}). Moreover a broad absorption band around 430 nm was generated due to the chalcone structure in its anionic form (CH^{3-}) (González et al., 2002; Montenegro et al., 2007). As a result, the solvent used for the dissolution of naringin and the pH could potentially influenced the interactions between chitosan and naringin or its chalcone isomer and its reactivity upon UV irradiation.

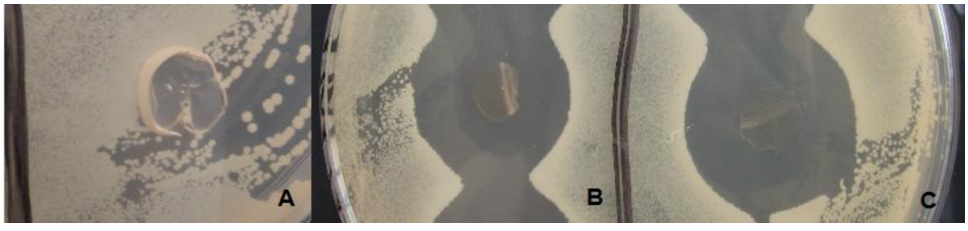

Fig. 4. Antimicrobial activity of chitosan based films against *L. innocua*: (A) Chitosan film; (B) CE-chitosan film; (C) CE-chitosan UV irradiated film.

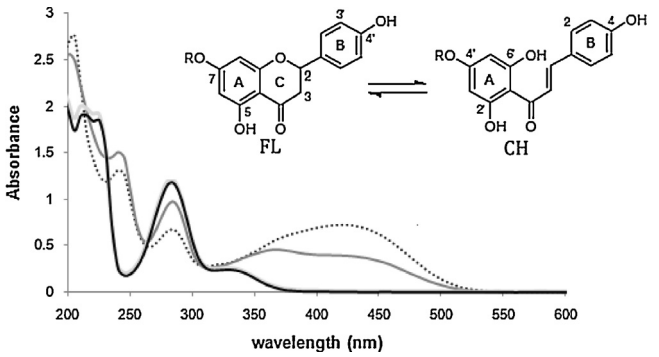

Fig. 5. Absorption spectra of Naringin at different concentrations of NaOH/different pH. (—): naringin in 0.1 mM NaOH/pH = 9.4; ( ): naringin in 1.0 mM NaOH/pH = 10.6; ( ): naringin in 0.01 M NaOH/pH = 11.9; (····): naringin in 0.1 M NaOH/pH = 12.9.

3.2.2. UV–vis characterization of N-chitosan films

The N-chitosan films prepared by mixing aqueous acetic acid solution of chitosan and naringin dissolved in ethanol solution displayed a broad absorption up to 470 nm (Fig. 6A). The same type of absorption pattern was observed when the sodium salt of naringin was used (Fig. 6B). This absorption is mainly due to the flavanone part of naringin with some contribution of the chalcone structure and their corresponding deprotonated entities partly formed by the action of some amino groups present in chitosan (*vide infra*).

Irradiation at 350 nm of N-chitosan films (concentration 0.05% w/v) either dissolved in ethanol (Fig. 6A) or in NaOH (Fig. 6B) leads to the formation of a new broad band centered at around 380 nm. As the new band appears approximately at the same wavelength in both cases, it can be suggested that the same compound is formed after irradiation regardless of the solvent used. After irradiating 6 h and storing the film in the dark for 24 h, a slight decrease on the absorption centered at 335 nm was noticeable. The chitosan part of the film contributes only for a minor part to the absorption at 310 nm. The band at 380 nm is likely due to the formation of 7-rhamnoglucosyl-5,4′-dihydroxyflavonol (Montenegro et al., 2007) (Fig. 1) from the triplet state of the ionized form of flavanone structure of naringin present in the chitosan film.

The slight decrease of absorption at 335 nm after one day dark reaction of 6 h-irradiated film (Fig. 6A and B) is likely ascribable to a protonation in the film of the anionic form of flavanone structure of naringin and of its possible flavonol photoproduct. When the concentration of naringin sodium salt was increased (from 0.05% to 0.1%) the chitosan films were much more stable against UV-light (mainly above 350 nm) after 6 h of irradiation (Fig. 6C). The increase of photostability is maybe only a matter of internal filter. As a matter of fact, the incorporation of naringin into chitosan films at appropriate concentration led to an improvement of the film barrier properties toward UV light.

In consequence, the high absorbance of N-chitosan films in the UV range can make these films excellent barriers to prevent the lipid oxidation induced by UV light when they are applied in food packaging systems.

3.2.3. Thermal characterization of N-chitosan films

The calorimetric analysis of pure naringin showed a sharp endothermic melting peak at around 130 °C (data not shown). The absence of the endothermic melting peak in the DSC thermogram of N-chitosan films suggested an interaction between the flavonoid and chitosan. In the same way, Ficarra et al. (2002) observed two endothermic peaks for physical mixtures of flavonoids and β-cyclodextrin and just one peak and the absence of the melting peak of the flavonoid when an inclusion complex was formed.

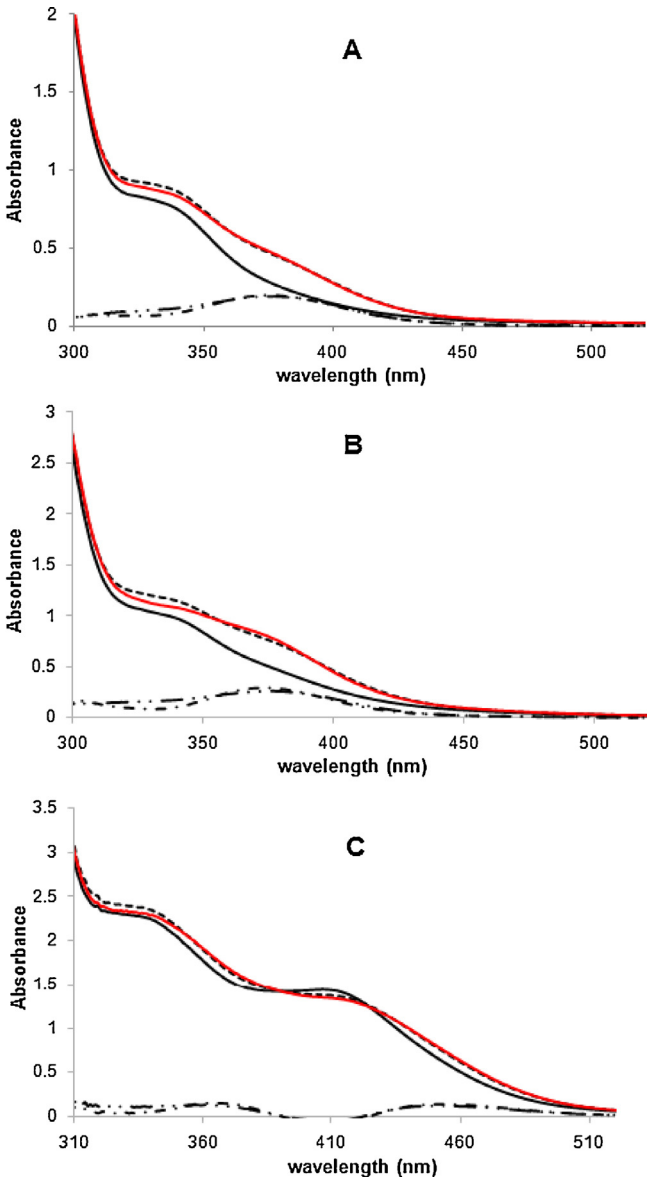

Fig. 6. UV–vis spectra of control and irradiated films made with chitosan (1% w/v) and naringin (0.05% w/v) in ethanol (A) and as sodium salt (B) and naringin (0.1% w/v) as sodium salt (C): (—) control; (– –) irradiated 6 h; ( ): irradiated 6 h then left in darkness for 24 h; (— · · —) absorption difference between films irradiated 6 h and control; (– · –) absorption difference between films irradiated 6 h then left in darkness for 24 h and control.

The differential scanning calorimetry (DSC) studies revealed a broad endothermic peak with a maximum centered at around 135–140 °C in all chitosan based films corresponding to water evaporation as already observed (Neto et al., 2005). No significant differences were observed in T_m values between different films regardless of UV treatment or naringin incorporation in the matrix (Table 1).

Table 1
Glass transition temperature and melting temperature for the different film samples. Results given as mean ± SD.

Film	T_g (°C)	T_m (°C)
Chitosan	54.22 ± 0.89	137.18 ± 2.90
Chitosan UV	86.84 ± 2.28	138.98 ± 6.92
N-chitosan	59.49 ± 0.35	140.97 ± 3.13
N-chitosan UV	61.17 ± 0.51	137.59 ± 1.02

Water molecules can be bound by hydroxyl and amino groups present in the chitosan structure (Neto et al., 2005), with stronger interaction with hydroxyl groups (Rueda, Secall, & Bayer, 1999). Thus, water molecules bound to amino groups could be removed easier (at lower temperatures) than those bound to hydroxyl groups. In this study, water evaporation started approximately at 85 °C for chitosan films, indicating that the water would be predominantly bound to the amino groups. For UV irradiated chitosan films, water evaporation started just after the T_g at approximately 100 °C, suggesting that the water was more strongly bound. In this case, amino groups could be less available to form hydrogen bonds with water molecules due to cross-links, as also observed by Neto et al. (2005). In the case of N-chitosan films, water evaporation started at around 95–100 °C, suggesting that water would be predominantly bound to hydroxyl groups. Interactions between naringin and chitosan could make amino groups less available for water binding.

Regarding T_g values, the incorporation of naringin into chitosan films made them less flexible (decrease in terms of chain mobility) due to interactions between chitosan and naringin. Besides, UV treated films (both with and without naringin) were more rigid (T_g value increased) when compared to the untreated ones. Therefore, UV treatment would induce cross-links between chitosan chains, thus decreasing their mobility. This effect was less pronounced in N-chitosan films probably due to the cross-links or interactions that already existed between chitosan and naringin.

3.2.4. Naringin release profile from chitosan-based films

A higher quantity of naringin was released from N-chitosan control films than from N-chitosan irradiated films (Fig. 7), which indicates stronger interactions between the matrix and naringin in the irradiated samples.

Besides, larger standard deviations were obtained in non-irradiated films compared to irradiated ones, which could imply a less homogeneous release of naringin between replicates and therefore worse repetitiveness between them.

After almost 24 h of immersion in the simulant (ethanol 95% v/v), 75% of total naringin in N-chitosan control films was released, whereas this percentage decreased to 48% in the case of irradiated samples. This could be due to different reasons: (i) more naringin was grafted to chitosan or the interactions between chitosan and naringin were stronger; or (ii) the irradiation treatment induced the cross-linking of chitosan, decreasing the release rate of naringin from the matrix. Such interactions in films prepared with N-carboxybutylchitosan and quercetin allowing the development of potential controlled release systems have been described by Dias et al. (2011).

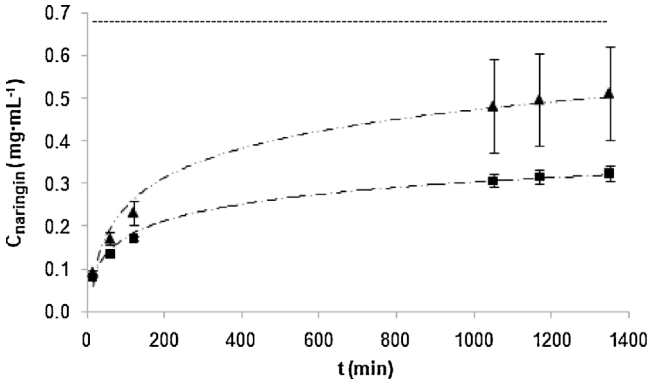

Fig. 7. Released naringin (mg mL^{-1}) from chitosan based films into 95% (v/v) ethanol: (▲) N-chitosan (1% w/v) films; (■) N-chitosan (1% w/v) films irradiated for 6 h. (- - -) dash line on the top represents the maximum theoretical concentration of naringin that could be released.

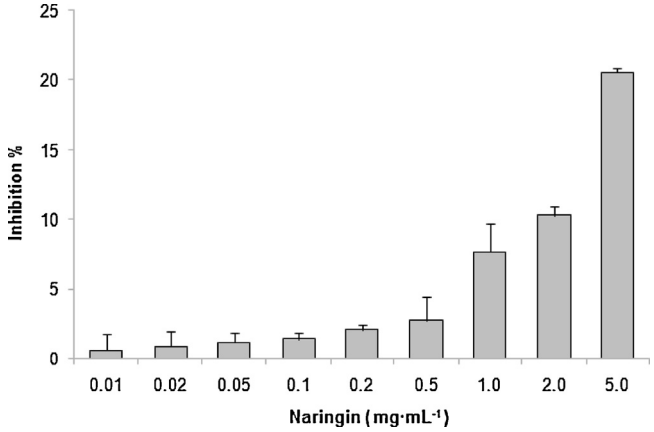

Fig. 8. Inhibition percentage of the free radical DPPH by standard solutions of naringin.

3.2.5. DPPH radical scavenging activity of naringin and released naringin solution

The antioxidant capacity was evaluated by plotting the concentrations of different standard solutions of naringin against the percentage of inhibition of free radical scavenging capacity of DPPH as shown in Fig. 8. It increased as the naringin concentration increased.

Nevertheless the antioxidant activity was limited because even with the highest concentration (5 mg mL^{-1}), less than 25% inhibition was achieved, in accordance with the very low antiradical activity of naringin observed by other authors (Burda & Oleszek, 2001).

The inhibition percentage of the simulant exposed to N-chitosan films was determined, using the same DPPH method. The antioxidant activity of the solution with the naringin released from N-chitosan control films was 5.04 ± 1.15, whereas it decreased to 2.88 ± 0.37 in the N-chitosan irradiated films. Thus, the antioxidant activity of naringin was retained but less naringin was released from N-chitosan irradiated films in agreement with the results obtained in Fig. 7, where higher amounts of naringin from N-chitosan control films were obtained.

3.2.6. Antimicrobial activity of N-chitosan films

Chitosan and N-chitosan films showed no inhibition zones around film disks against *L. innocua*. Nevertheless, growth inhibition under the materials was observed for both non-irradiated and irradiated chitosan films and for some of the formulations of N-chitosan films (data not shown). The antimicrobial activity of chitosan is generally considered to be due to its positively charged amino group. Therefore, a decrease in the antimicrobial activity of N-chitosan films was expected as UV–vis and DSC results showed interactions (ionic, H-bonding or even covalent bonding) between the amino group of chitosan and naringin, making less -NH_2 groups available to be protonated and impart its antimicrobial activity. Sousa et al. (2009) observed a reduction of the antimicrobial effect against *Staphylococcus aureus* for chitosan functionalized with catechin, since about 50% of available deprotonated amino groups on the chitosan fibers were bound to catechin. Besides, Mayachiew et al. (2010) observed no inhibition zone for galangan extract-chitosan films when extract concentration was 0.3% (w/w), proposing that the interactions between chitosan and the extract limited the diffusion of the extract.

4. Conclusions

CE-chitosan films showed antimicrobial activity against *L. innocua*, which was maintained even after exposing the film to UV irradiation for 6 h. Consequently, these kinds of films could be useful to enhance the safety of foodstuff and for protecting them from UV light deterioration, maintaining the quality of food products, as absorbance of UV light after incorporation of citrus extract into chitosan films was greatly enhanced.

Ultraviolet–visible spectra of N-chitosan films suggested the formation of a photoproduct of naringin after irradiation and storage in darkness, which was independent of the solvent used. Increasing the concentration of naringin in the films (from 0.05% to 0.1%) made the films more stable against UV-light irradiation.

Thermal characterization showed that UV treated films, both with and without naringin, were more rigid compared to the untreated ones. Besides, the incorporation of naringin into chitosan films retarded the temperature at which water evaporation started, suggesting an interaction between chitosan amino group and naringin. However, further studies should be carried out to establish the exact nature of this interaction. On the other hand, UV treatment seemed to establish cross-links between chitosan chains in the films. The prepared films proved to have antimicrobial activity by direct contact with the agar medium due to chitosan antimicrobial effectiveness. Although more extensive photochemical studies and further tests would be needed to exactly elucidate the photoproducts formed and their percentage upon irradiation, it seems clear that the film could potentially act as a barrier between the food and the light.

Acknowledgements

This work has been performed within an Aquitaine-Basque Country Cooperation project. This paper is contribution nr. 656 from AZTI-Tecnalia (Marine or Food Research). L. Iturriaga acknowledges the Department of Agriculture, Fisheries and Food of the Basque Government for the fellowship. A. Lafaille is also gratefully acknowledged for the antimicrobial tests.

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