



SCREENING OF THE PHYSICAL AND STRUCTURAL PROPERTIES OF CHITOSAN-POLYCAPROLACTONE FILMS ADDED WITH *Moringa oleifera* LEAF EXTRACT

ANÁLISIS DE LAS PROPIEDADES FÍSICAS Y ESTRUCTURALES DE LAS PELÍCULAS DE QUITOSANO-POLICAPROLACTONA ADICIONADAS CON EXTRACTO DE HOJA DE *Moringa oleifera*

J.A. Núñez-Gastélum^{1*}, J.R. Rodríguez-Núñez², L.A. de la Rosa¹, A.G. Díaz-Sánchez¹, E. Alvarez-Parrilla¹, Alejandro Martínez-Martínez¹, G. Villa-Lerma³

¹Departamento de Ciencias Químico-Biológicas, Instituto de Ciencias Biomédicas, Universidad Autónoma de Ciudad Juárez, Anillo envolvente del PRONAF y Estocolmo s/n, Ciudad Juárez, Chihuahua, 32310, México.

²Programa de Biotecnología, División de Ciencias de la Salud e Ingenierías, Universidad de Guanajuato Campus Celaya-Salvatierra, Mutualismo #303, Colonia La Suiza, Celaya, Guanajuato, 38060, México.

³Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora, 5 de Febrero #818 Sur, Ciudad Obregón, Sonora, 85000, México.

Received March 14, 2018; Accepted August 12, 2018

Abstract

Physico-structural properties of biodegradable films of chitosan and polycaprolactone (PCL) added with *Moringa oleifera* leaf extract were determined. The films were analyzed for thickness, transparency, solubility, Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). The transparency was increased as the PCL concentration increased and there was no difference in solubility for any of the formulations evaluated. On the other hand, FTIR spectra revealed an increase in hydroxyl groups, amides and carbonyls bands as the concentration of PCL and *M. oleifera* extract increased. XRD diffractograms showed a good miscibility between chitosan and PCL using concentrated acetic acid as the solvent. Films obtained could be used as biodegradable packing, besides it is possible to add natural extracts to the films that could work as additives.

Keywords: chitosan, polycaprolactone, *Moringa oleifera*, biodegradable films, food packaging.

Resumen

Se determinaron las propiedades físico-estructurales de películas biodegradables a base de quitosano y policaprolactona (PCL) añadidas con extracto de hoja de *Moringa oleifera*. Las películas se analizaron en cuanto a grosor, transparencia, solubilidad, espectroscopia infrarroja con transformada de Fourier (FTIR) y difracción de rayos X (XRD). Los valores de transparencia incrementaron a medida que aumentaba la concentración de PCL, además no existió diferencia en la solubilidad para ninguna de las formulaciones evaluadas. Por otro lado, los espectros obtenidos por FTIR revelaron un aumento en los grupos hidroxilo, amidas y bandas de carbonilos a medida que aumentaba la concentración de PCL y del extracto de *M. oleifera*. Los difractogramas XRD mostraron una buena miscibilidad entre quitosano y PCL usando ácido acético concentrado como disolvente. Las películas obtenidas podrían usarse como empaques biodegradables, además de que es posible agregar extractos naturales a las películas que podrían funcionar como aditivos.

Palabras clave: quitosano, policaprolactona, *Moringa oleifera*, películas biodegradables, empaques alimentarios.

1 Introduction

The growing environmental awareness has led to the need of reducing the use of non-biodegradable materials derived from petroleum and in turn has promoted the use of biodegradable materials from renewable sources (Abdollahi *et al.*, 2012; Aider, 2010). So that the use of biofilms for food packaging is a promising alternative to reduce the

environmental impact and to ensure food quality. Several authors have pointed out that the main biopolymers used for the development of this technology are cellulose, chitosan, starch and alginates (Pérez-Orozco *et al.*, 2011; Rodríguez-Núñez *et al.*, 2012).

Chitosan (CH) is a cationic polysaccharide (poly- β -1-4-amino-deoxy-D-glucan) derived from chitin, which is the second most abundant biopolymer in nature, after cellulose (Wu *et al.*, 2016).

* Corresponding author. E-mail: jose.nunez@uacj.mx

It is obtained from the thermo-alkaline deacetylation of chitin and it is considered as a promising material for application in several areas such as the food, cosmetic, biomedical, agricultural and chemical industry. CH has characteristics such as being, biodegradable, biocompatible, antioxidant, non-toxic, and antimicrobial which makes it a highly important material for the development of active packaging and coatings (Rubentheren *et al.*, 2015; Qui *et al.*, 2014; Rodríguez-Núñez *et al.*, 2012).

On the other hand, PCL is a semi-crystalline polyester obtained from the polymerization of ϵ -caprolactone. This polymer has the characteristic of being biodegradable, biocompatible, non-toxic, adherent, crystallizes easily and it has a melting point of 59–64 °C. Moreover, PCL is approved by FDA for medical applications and due to its miscibility in organic solvents, it is possible to form blends with other polymers including biopolymers (Peña *et al.*, 2016; Mina *et al.*, 2013). In addition, it has been mixed with starch to obtain biofilms for packaging, improving the processability (Pacheco *et al.*, 2014).

Nowadays, there is an increasing demand for products including natural compounds as an alternative to products containing chemical additives (Demetri *et al.*, 2016). These compounds are often incorporated into biofilms, either in pure form or as an extract from plant matrices, offering them antimicrobial and antioxidant properties, and even influencing their mechanical, optical, and barrier properties (Rodríguez-Núñez *et al.*, 2014; Hernández-Ochoa *et al.*, 2011).

Recently, the antioxidant and antimicrobial properties of *Moringa oleifera* leaf have been reported, and these properties are attributed to the presence of ascorbic acid, flavonoids, polyphenolic compounds, carotenoids, essential amino acids and high concentrations of minerals such as iron, calcium and potassium (Zhao and Zhang, 2013; Jayawardana *et al.*, 2015). Also, isolated peptides and low molecular weight proteins from *M. oleifera* leaves presented antibacterial and antifungal capacity (Dahot *et al.*, 1998).

So far there is not much information on the use of CH/PCL mixtures. There are reports of the preparation of

nanofibers, hydrogels, and films for biomedical applications (Sahoo *et al.*, 2010; Bhattarai *et al.*, 2009). Only two studies evaluate the applicability of this mixture of polymers for food packaging using chloroform for PCL dissolution (van den Broek *et al.*, 2015; Joseph *et al.*, 2011). However, the use of toxic organic solvents is not desirable for food purposes and none of the aforementioned studies evaluate the physical-structural effect of a bioactive compound or extract on the CH/PCL mixture. This is why the aim of the present work is to investigate the physical-structural properties of CH/PCL films added with *M. oleifera* leaf extract for its possible application as food packaging.

2 Materials and methods

2.1 *M. oleifera* leaf extract

All reagents were obtained from Sigma (St. Louis, MO, USA), unless otherwise indicated. Freeze-dried *M. oleifera* leaves were extracted using a hydroalcoholic mixture as a solvent. Specifically, 50 mg of sample was placed in a centrifuge tube and 10 mL of aqueous methanol at 80% was added and sonicated (40 kHz) for 30 min in dark. The extract was centrifuged (2000 g) for 30 min at 4 °C and the supernatant was collected. The residues were re-extracted under the same conditions; finally, both supernatants were combined, freeze-dried and stored at –80 °C for later use (Núñez-Gastélum *et al.*, 2015).

2.2 Preparation of biodegradable films

Nine films was prepared following the method of solvent evaporation (Table 1). The characteristics of the chitosan used were: molecular weight 50,000-190,000 Da, 75-85% of deacetylation degree and 20-300 cP of viscosity (1 wt.% in 1% acetic acid at 25 °C). Formulations were made using mixtures of CH / PCL at two ratios (90:10 and 80:20), sum of polymers in the solutions was 1% (w/v).

Table 1. Values of thickness, transparency and %WS obtained of the biodegradable films prepared with chitosan, PCL and MOE.

Treatment	Thickness (mm)	Transparency	%WS
CH	0.03 ± 0.01 ^a	2.25 ± 0.01 ^a	37.79 ^a
CH/MOE (500)	0.03 ± 0.01 ^a	3.18 ± 0.01 ^a	33.53 ^a
CH/MOE (1000)	0.03 ± 0.01 ^a	3.69 ± 0.01 ^a	37.66 ^a
CH/PCL (90:10)	0.03 ± 0.01 ^a	9.70 ± 0.07 ^{bc}	40.39 ^a
CH/PCL/MOE (90:10:500)	0.04 ± 0.01 ^{ab}	6.70 ± 0.05 ^b	61.19 ^a
CH/PCL/MOE (90:10:1000)	0.05 ± 0.02 ^b	6.04 ± 0.05 ^b	49.72 ^a
CH/PCL (80:20)	0.03 ± 0.01 ^a	16.40 ± 0.06 ^d	47.04 ^a
CH/PCL/MOE (80:20:500)	0.04 ± 0.01 ^{ab}	9.94 ± 0.06 ^{bc}	34.68 ^a
CH/PCL/MOE (80:20:1000)	0.04 ± 0.01 ^{ab}	14.22 ± 0.15 ^{cd}	47.57 ^a

For thickness and transparency values are reported as the mean ± standard deviation, different letters in the same column are significantly different Tukey mean test ($\alpha = 0.05$).

Aqueous acetic acid (90%) was used as solvent and *M. oleifera* extract (MOE) was added at concentrations of 0, 500 and 1000 ppm. Specifically, 100 ml of each formulation was prepared as follow: CH, PCL and MOE were weighed and placed in a vessel, then 80 ml of the acetic acid solution was added, this mixture was stirred for 4 h at 150 rpm finally acetic acid was added until 100 ml were reached. The films were cast onto a polypropylene plate and dried at 40 °C for 24 h. Finally, in order to neutralize the pH of the films, they were immersed in a 1.5 M Tris-Base buffer solution for 30 s and dried in an oven at 40 °C for 24 h (Rodríguez-Núñez *et al.*, 2014).

2.3 Physical properties

Transparency and thickness were determined following the procedure proposed by Rodríguez-Núñez *et al.* (2014). For thickness measures, an electronic vernier was used to analyze the thickness of the films, 7 random samplings were made for each film. Subsequently, the mean (mm) and standard deviation of the data collected were calculated. For transparency measures, films were cut into 6×3 cm rectangles and placed on the inner side of a spectrophotometric cell (UV-Visible Spectrophotometer, Varian, Cary 50 Bio, Palo Alto, CA, USA). Absorbance was determined at 600 nm (A_{600}) and then divided by the thickness (Th) of each one, transparency values were determined according to the following equation: Transparency = A_{600}/Th . The measurements were made in triplicate for each film and its average was reported. Percentage of solubility in water (%WS) was determined following the method proposed by Bourtoom and Chinnan (2008), with some modifications. Dried films were weighed (W_o) and immersed in distilled water at room temperature (25 ± 1 °C) until equilibrium was reached (1 h), then dried at 60 °C for 24 h. Finally, the dry weight of the films (W_d) was recorded and %WS was calculated based on the following equation: %WS = $([W_o - W_d]/W_o) \times 100$. Samples of each formulation were analyzed in triplicate.

2.4 Structural analysis

These properties were determined following the procedure proposed by Rodríguez-Núñez *et al.* (2014). FTIR spectra of the samples were acquired using a spectrophotometer Midac equipped with an accessory of attenuated total reflectance (ATR) (GBC, Dandenong, Australia). The spectra were collected by averaging 100 scans at 4000–650 cm^{-1} spectral resolution. The technique applied was by attenuated total reflectance (ATR) by coupling a multi-rebound avatar accessory with 45° ZnSe crystal. X-ray diffraction patterns (XRD) were performed using a Xpert Pro PANalytical diffractometer with an incident radiation $\text{CuK}\alpha$ and wave length of $\lambda = 1.5418$ Å operated at 35 kV y 25 mA. A scan was performed with a rotator at 15 rpm (with background value equal to zero), the sample container was

made of silicon in order to avoid any interference with the environment, and it was worked at room temperature. Measurements were performed in the range of $2\theta = 5-60$ °C with a time lapse of 8 s and steps of 0.02°.

2.5 Statistical analysis

One-way analysis of variance in a completely randomized design and Tukey's mean test ($\alpha = 0.05$) was applied to the data. Due to the heterogeneity of variances in the different treatments for %WS variable, non-parametric Kruskal-Wallis test, and the Z-based mean range test was used. All tests were performed using SAS version 9.3 (SAS Institute).

3 Results and discussion

3.1 Thickness and transparency

The values of thickness and transparency of the CH/PCL formulations added with MOE (CH/PCL/MOE) are shown in Table 1. It is noteworthy that thickness and transparency of neat chitosan (CH) and (CH/MOE) films showed no significant difference between them ($p \leq 0.05$). However, a significant increase ($p > 0.05$) in the transparency values was observed when PCL was added. Increasing the concentration of PCL has a strong impact on transparency values; this minimizes the negative effect of the extracts on this property. These results are attributed to the nucleating action of chitosan in the formulation, which prevents the formation of amorphous areas in PCL, increasing transparency. High concentrations of nucleating agents block the formation of crystals in PCL, reducing crystallinity degree due to an increased nucleation (Cesur and Kahraman, 2016). It has been reported that the chitin concentration significantly affected the crystallization temperature of PCL in mixtures of PCL/chitin, needing a higher temperature to achieve crystallization and demonstrating the nucleating effect of chitin (Martínez *et al.*, 2008).

3.2 Percentage of solubility in water

%WS values showed no significant difference ($p \leq 0.05$) between treatments (Table 1). Several authors point to the hydrophobic character of PCL, however, PCL concentrations were not high enough to alter the continuous phase of the chitosan film (Sahoo *et al.*, 2010). In addition, high concentrations of additives increase the solubility values of PCL due to the interference with crystallization degree; this may be related to the non-significant increase ($p \leq 0.05$) in the solubility values of most of the formulations containing it (Cesur and Kahraman, 2016). Films based on mixtures of CH/Sorbitol had solubility values higher than 50% in chitosan formulations added with glycerol and

sorbitol, founding that pure chitosan films and 20% sorbitol formulations showed a complete dissolution (Rodríguez-Núñez *et al.*, 2014).

These values are similar to those reported in the present work, however, none of the formulations showed a solubility of 100%. It should be noted that chitosan is soluble in water at $\text{pH} \leq 6.5$ due to the protonation of the amino group causing the cationic charge to the polymer (Shu and Zhu, 2002). In this sense, the low solubility obtained in the present investigation may be related to the neutralization treatment applied to the formulations, which neutralized the remaining acetic acid in the films, avoiding the acidification of the aqueous medium, which would cause an increase in the solubility of chitosan. Likewise, films showed a similar solubility range to those formulated with essential oils, which reduced the values up to 24%, reported with cinnamon oil (Ojagh *et al.*, 2010).

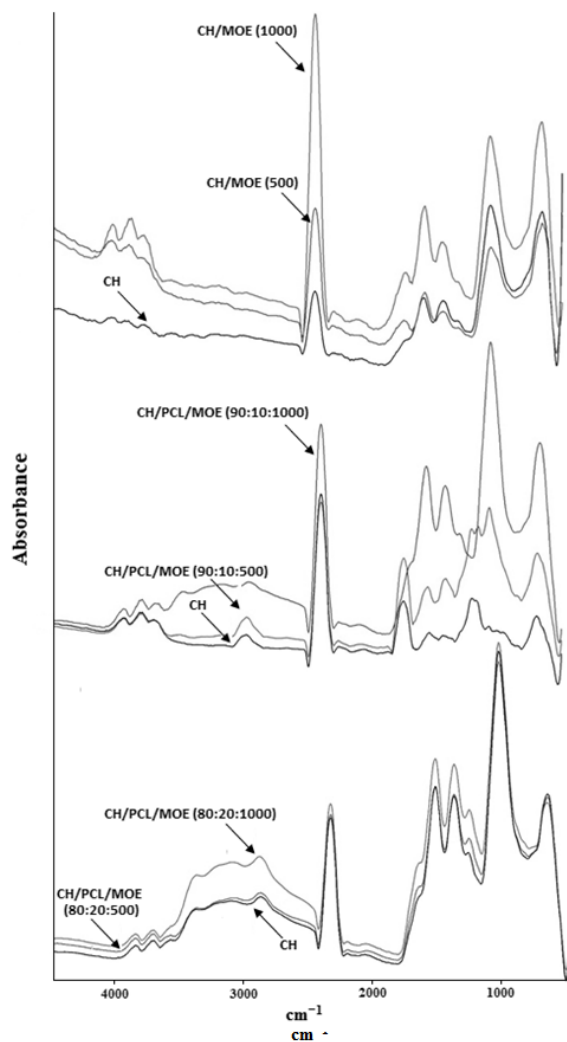


Fig. 1. FTIR spectra of films prepared with different formulations of CH, PCL and MOE.

Also, a reduction up to 63% in water vapor permeability values by adding 30% citronella oil was observed (Shen and Kandem, 2015). Based on the obtained results, it would be feasible to apply a neutralization process to films added with essential oils to increase water barrier properties.

3.3 Infrared spectroscopy

The results of FTIR can be observed in Figure 1. The typical absorption bands of chitosan can be observed at 3600 to 3000 cm^{-1} , corresponding to the groups O–H and N–H in symmetrical/asymmetrical tension attributed to hydrogen bonds. In this region an increase in the intensity of the bands was observed when adding MOE, this indicates an interaction by means of hydrogen bonds of flavonoids and flavonols with chitosan. Similarly, it can be observed an increase in the intensity of the band at 1750 cm^{-1} corresponding to the carbonyl group ($\text{C}=\text{O}$) which indicates the presence of esters from organic acids contained in the MOE as well as the residues of acetic acid. The presence of the pyranoside structures (chitosan and other carbohydrates) is confirmed by the signal at 1150 cm^{-1} , which is an indicator of the vibrations of the glycosidic bonds ($\text{C}-\text{O}-\text{C}$) (Rodríguez-Núñez *et al.*, 2014; Corazzari *et al.*, 2015). Finally, the bands of amide I, II and III, which are typical of chitosan, are observed at 1650, 1590 and 1317 cm^{-1} , respectively (Branca *et al.*, 2016). The FTIR spectra obtained with the CH/PCL mixtures showed bands at 2949 and 2865 cm^{-1} regions corresponding to the symmetric and asymmetric tension of the $-\text{CH}_2$ groups from PCL. In addition, bands observed between 1157 and 1170 cm^{-1} corresponds to the overlap of the symmetrical tension of ether groups and tension of $\text{C}-\text{C}$ and $\text{C}-\text{O}$ on the amorphous phase of the PCL, on the vibrations of the structure of pyranose (Elzein *et al.*, 2004; Corazzari *et al.*, 2015). The most significant changes are observed in the amide region (1500–1600 cm^{-1}) due to the possible interaction of chitosan with the PCL and the polyphenolic compounds of the MOE, specifically with their carbonyl groups. These effects were observed when mixing PCL with starch (Ortega-Toro *et al.*, 2016). Finally, homogeneous bands can be observed in Figure 1C, which may be related to a high concentration of PCL (20%) that allows peaks corresponding to the carbonyl group of the PCL in the 1728 cm^{-1} region to predominate.

3.4 X-ray diffraction

The XRD patterns of the different formulations are shown in Figure 2. If the biopolymers are immiscible or have low compatibility, then the crystalline regions corresponding to each biopolymer must be observed separately (Abdelrazek *et al.*, 2016). It is possible to observe a characteristic broad band in all formulations, this band at $2\theta = 20^\circ$ is typical for the crystalline arrangement of chitosan (Rodríguez-Núñez *et al.*, 2014).

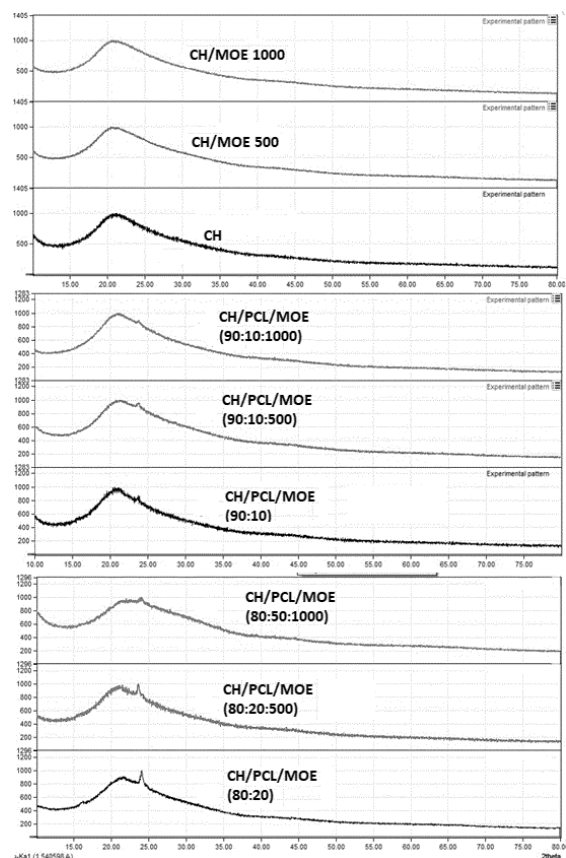


Fig. 2. XRD patterns of films obtained using CH, PCL and MOE.

Formulations added with PLC showed an additional band at $2\theta = 24^\circ$. The typical XRD pattern of pure PCL has two bands, at $2\theta = 21.4^\circ$ and at $2\theta = 23.8^\circ$ (Abdelrazek *et al.*, 2016). These bands are indexed to (110) and (200) planes of an orthorhombic crystal structure (Salmoria *et al.*, 2016). In this work it is possible to observe the absence of the band at $2\theta = 21.4^\circ$ in formulations with PCL, in addition the band at approximately $2\theta = 23.8^\circ$ is very weak.

These results can be related to good miscibility between chitosan and PCL using concentrated acetic acid as solvent. It is possible to observe changes in the band intensity at $2\theta = 23.8^\circ$ between formulations with different concentrations of PCL. Also, a decrease in the band intensity is observed as the MOE concentration increases. It has been reported a decrease in the intensity of the characteristic bands of PCL when mixed with silver sulfadiazine due to the interactions of the drug with the polymer chains, which is similar to those obtained in the present work (Salmoria *et al.*, 2016). A similar effect was observed when plasticizing agents were added to the chitosan, demonstrating a decrease in peak intensity with increasing concentration (Rodríguez-Núñez *et al.*, 2014).

Conclusions

It is possible to combine chitosan with PCL using acetic acid as solvent without the use of chemical solvents. The physical and structural properties of the films were maintained showing good miscibility, an increase of crystalline regions by increasing PCL concentrations and the interactions were between amides, carboxyls and hydroxyls groups. The concentration of MOE did not affect water solubility of the films, therefore, it did not affect the polarity characteristics of the formulations. Chitosan-PCL Films obtained could be used as biodegradable packing, besides it is possible to add natural extracts to the films that could work as additives.

Acknowledgements

J.A.N.-G. is grateful to “Programa para el Desarrollo Profesional Docente (PRODEP)” for funding the project: “Apoyo a la Incorporación de Nuevos PTC (No. UACJ-PTC-311)”. J.R.R.-N. acknowledge to Universidad de Guanajuato the support with the project CIFOREA 012/2015. Thanks to the technical assistance of B.S. in chemistry Jonathan Clemente and the collaboration of Dr. Bonifacio Alvarado in the X-ray measurements and reagents provided.

Abbreviations

%WS	percentage of solubility
ATR	attenuated total reflectance
CH	chitosan
FTIR	Fourier transform infrared spectroscopy
MOE	<i>M. oleifera</i> extract
PCL	polycaprolactone
W_d	weight of the films after %WS test
W_o	weight of dried films
XRD	X-ray diffraction

References

- Abdelrazek, E. M., Hezma A. M., El-kodhari, A., and Elzayat, A. M. (2016). Spectroscopic studies and thermal properties of PCL/PMMA biopolymer blend. *Egyptian Journal of Basic and Applied Science* 3, 10-15.
- Abdollahi, M., Rezaei, M., and Farzi, G. (2012). Improvement of active chitosan film properties with rosemary essential oil for food packaging. *International Journal of Food Science & Technology* 47, 847-853.

- Aider, M. (2010). Chitosan application for active bio-based films production and potential in the food industry: Review. *LWT - Food Science and Technology* 43, 837-842.
- Bhattarai, N., Zhensheng, L., Gunn, J., Leung, M., Cooper, A., Edmondson, E., Veiseh, O., Chen, M.-H., Zhang, Y., Ellenbogen, R. G., and Zhang, M. (2009). Natural-synthetic polyblend nanofibers for biomedical applications. *Advanced Materials* 21, 2792-2797.
- Bourtoom, T., and Chinnan, M. S. (2008). Preparation and properties of rice starch-chitosan blend biodegradable film. *LWT-Food Science and Technology* 41, 1633-1641.
- Branca, C., D'Angelo, G., Crupi, C., Khouzami, K., Rifici, S., Ruello, G., and Wanderlingh, U. (2016). Role of the OH and NH vibrational groups in polysaccharide-nanocomposite interactions: A FTIR-ATR study on chitosan and chitosan/clay films. *Polymer* 99, 614-622.
- Cesur, S., and Kahraman, T. (2016). Printing properties of polycaprolactone composite films. *Progress in Organics Coatings* 98, 10-13.
- Corazzari, I., Nisticò, R., Turci, F., Faga, M. A., Franzoso, F., Tabasso, S., and Magnacca, G. (2015). Advanced physico-chemical characterization of chitosan by means of TGA coupled on-line with FTIR and GCMS: Thermal degradation and water adsorption capacity. *Polymer Degradation and Stability* 112, 1-9.
- Dahot, M. U. (1998). Antimicrobial activity of small protein of *Moringa oleifera* leaves. *Journal of the Islamic Academy Sciences* 11, 27-32.
- Demitri, C., De Benedictis, V. M., Madaghiele, M., Corcione, C. E., and Maffezzoli, A. (2016). Nanostructured active chitosan-based films for food packaging applications: Effect of graphene stacks on mechanical properties. *Measurement* 90, 418-423.
- Elzein, T., Nasser-Eddine, M., Delaite, C., Bistac, S., Dumas, P. (2004). FTIR study of polycaprolactone chain organization at interfaces. *Journal of Colloid Interface Science* 273, 381-387.
- Joseph, S., Prashanth, K. V. H., Rastogi, N. K., Indiramma, A. R., Reddy, S. Y., and Raghavarao, K. S. M. S. (2011). Optimum blend of chitosan and poly-(ϵ -caprolactone) for fabrication of films for food packaging applications. *Food and Bioprocess Technology* 4, 1179-1185.
- Hernández-Ochoa, L., Gonzales-Gonzales, A., Gutiérrez-Mendez, N., and Quintero-Ramos, A. (2011). Study of the antibacterial activity of chitosan-based films prepared with different molecular weights including spices essential oils and functional extracts as antimicrobial agents. *Revista Mexicana de Ingeniería Química* 10, 455-463.
- Martínez, G., Matos, M., Sabino, M., Urbina de Navarro, C., Barrios, C., Taddei, A., Sajo, C., Arnal, M. L., and Müller, A. (2008). Estudio de una mezcla binaria biodegradable: Policaprolactona/quitina. *Revista Iberoamericana de Polímeros* 9, 313-321
- Mina, J. H., Valadez-González, A., Herrera-Franco, P., Zuluaga, F., and Delvasto, S. (2013). Preparation and physical-chemical and mechanical characterization of ternary blends of polylactide (PLLA), polycaprolactone (PCL) and thermoplastic starch (TPS). *Revista Latinoamericana de Metalurgia y Materiales* 33, 82-91.
- Núñez-Gastélum, J. A., Alvarez-Parrilla, E., de la Rosa, L. A., Martínez-Ruiz, N. R., González-Aguilar, G., and Rodrigo-García, J. (2015). Effect of harvest date and storage duration on chemical composition, sugar and phenolic profile of 'Golden Delicious' apples from northwest Mexico. *New Zealand Journal of Crop and Horticultural Science* 43, 214-221.
- Ojagh, S. M., Rezaei, M., Razavi, S. H., and Hosseini, S. M. H. (2010). Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. *Food Chemistry* 122, 161-166.
- Ortega-Toro, R., Muñoz, A., Talens, P., and Chiralt, A. (2016). Improvement of properties of glycerol plasticized starch films by blending with a low ratio of polycaprolactone and/or polyethylene glycol. *Food Hydrocolloids* 56, 9-19.
- Pacheco, G., Flores, N. C., and Rodríguez-Sanoja, R. (2014). Bioplásticos. *BioTecnología* 18, 27-36.
- Peña, J. A., Gutiérrez, S. J., Villamil, J. C., Agudelo, N. A., and Pérez, L. D. (2016). Polycaprolactone/polyvinylpyrrolidone/siloxane hybrid materials: Synthesis and *in vitro* delivery of diclofenac and biocompatibility with periodontal ligament fibroblasts. *Materials Science and Engineering: C* 58, 60-69.
- Pérez-Orozco, J. P., Barrios-Salgado, E., Róman-Guerrero, A., and Pedroza-Islas, R. (2011). Interaction of mesquite gum-chitosan at the interface and its influences on the stability of multiple emulsions $W_1/O/W_2$. *Revista Mexicana de Ingeniería Mexicana* 10, 487-499.
- Rodríguez-Núñez, J. R., López-Cervantes, J., Sánchez-Machado, D. I., Ramírez-Wong, B., Torres-Chávez, P., and Cortez-Rocha, M. O. (2012). Antimicrobial activity of chitosan-based films against *Salmonella typhimurium* and *Staphylococcus aureus*. *International Journal of Food Science & Technology* 47, 2127-2133.
- Rodríguez-Núñez, J. R., Madera-Santana, T. J., Sánchez-Machado, D. I., López-Cervantes, J., and Soto, H. V. (2014). Chitosan/hydrophilic plasticizer-based films:

- Preparation, physicochemical and antimicrobial properties. *Journal of Polymers and the Environment* 22, 41-51.
- Rubentheren, V., Ward, T. A., Chee, C. Y., and Tang, C. K. (2015) Processing and analysis of chitosan nanocomposites reinforced with chitin whiskers and tannic acid as a crosslinker. *Carbohydrate Polymers* 115, 379-387.
- Sahoo, S., Sasmal, A., Nanda, R., Phani, A. R., Nayak, P. L. (2010). Synthesis of chitosan–polycaprolactone blend for control delivery of ofloxacin drug. *Carbohydrate Polymers* 79, 106-113.
- Salmoria, G. V., Paggi, R. A., and Kanis, L. A. (2016). Manufacturing of PCL/SAG tubes by melt-extrusion for nerve regeneration: Structure and mechanical properties. *Polymer Testing* 55, 160-165.
- Shen, Z., and Kandem, D. P. (2015). Development and characterization of biodegradable chitosan films containing two essential oils. *International Journal of the Biological Macromolecules* 74, 289-296.
- Shu, X. Z., and Zhu, K. J. (2002). The influence of multivalent phosphate structure on the properties of ionically cross-linked chitosan films for controlled drug release. *European Journal of Pharmceutics and Biopharmaceutics* 54, 235-243.
- van den Broek, L. A. M., Knoop, R. J. I., Kappen, F. H. J., and Boeriu, C. G. (2015). Chitosan films and blends for packaging material. *Carbohydrate Polymers* 116, 237-242.
- Wu, C., Tian, J., Li, S., Wu, T., Hu, Y., Chen, S., Sugawara, T., and Ye, X. (2016). Structural properties of films and rheology of film-forming solutions of chitosan gallate for food packaging. *Carbohydrate Polymers* 146, 10-19.
- Zhao, S., and Zhang, D. (2013). Supercritical fluid extraction and characterization of *Moringa oleifera* leaves oil. *Separation and Purification Technology* 118, 497-502.