

Fabrication of Gelatin-based natural nanocomposite films using nanoclay and Chitosan for food packaging applications

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Abstract

Solid waste disposal and accumulation from various packaging materials made of synthetic polymers have led to intensified environmental concerns during the last decades. However, extensive research has been conducted on bio-nanocomposites as an alternative material for synthetic polymers in packaging applications during recent years. In this study, gelatin-based films modified with nano-clay and chitosan are proposed for food packaging. Nano-clay is used to improve various features such as mechanical strength and water vapor barrier properties. Also, chitosan is added to the gelatin matrix for anti-microbial and anti-fungal properties. The as-prepared nanocomposite films were characterized using FTIR, XRD and SEM techniques. Also, tensile strength, transparency, swelling behavior, water contact angle and permeability tests were performed. Based on the results, increasing nano-clay up to 5 wt. %, results in 25% tensile strength enhancement in the nano-composite film. Higher nano-clay contents led to strength decline. Elongation at break is reduced by increasing the nano-clay content from 25% to 40%. The contact angle was also reduced by increasing the nano-clay content from 95° to 90° showing a small hydrophobicity reduction.

Keywords: Bio-degradability; Chitosan; Gelatin; Nano-clay; Nanocomposite Film; Packaging.

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INTRODUCTION

Food packaging plays a critical role in food preservation from contaminants throughout the distribution chain which leads to shelf-life enhancement [1]. As a result, food packaging industry has been developed as one of the most important industries in the world constituting ~ 2 % of gross domestic product (GDP) in developed countries [2]. During recent decades, utilization of synthetic polymers for food packaging has been increased significantly in comparison with classical materials such as paper, glass and metals due to advantages such as high processability, low cost and weight and superior barrier properties toward water vapor and other concerned gases. Meanwhile, accumulation of packaging waste in the environment is emerged as a major environmental issue; 8 M tons of plastics are released in the oceans annually [3].

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Replacement of synthetic polymers with natural materials has recently been considered as a solution to the upcoming environmental problems. Various natural polymers have been utilized for packaging applications including starch [4], cellulose and its derivatives [5], gelatin [6], chitosan [7], agar [8] and copolymers such as poly(lactic acid) (PLA) [9], poly(caprolactone) (PCL) [10], poly(hydroxyalkanoate) (PHA) [11] and various blends [12]. Availability, low cost and biodegradability, has provoked extensive studies on the application of natural polymers in food packaging. However, intrinsic shortcomings such as rigidity, low barrier properties especially toward water vapor and also low distortion temperatures have limited their industrial development. Although, several studies have been performed on the improvement of natural polymer film properties like chemical modification, addition of plasticizers for flexibility, blending of various

polymers and addition of compatibilizers, the natural polymer-based films are still far from ideal in many food packaging applications.

Among various natural polymers, gelatin is obtained via controlled acidic or basic hydrolysis of collagen [13] with excellent film forming and high oxygen barrier properties making it a good alternative for synthetic polymers. However, weak resistance towards water vapor is among the limitations hindering the vast application of gelatin for food packaging. Shortcomings of gelatin can be somehow improved by blending with various polymers like chitosan resulting in higher hydrophobicity and higher water vapor resistance [14]. Also, due to the transmission of various diseases from food, addition of chitosan to gelatin can be helpful in preventing bacterial and fungal growth improving food preservation capacity due to antimicrobial activity and low toxicity [15-17].

During recent years, several studies have been performed on the improvement of synthetic and natural polymeric films using nanomaterials such as nano-clay [18, 19], nano-cellulose [12, 20] and nano-silver [21] which are utilized in various fields such adsorption [22], sensors [23] and packaging. Addition of nanoscale fillers can result in films with enhanced properties due to the improved interaction between the filler and the polymeric matrix [24]. Recently, packaging industry has focused on inorganic materials like clays and silicates as fillers. Nano-clays are 2D nano-plates with nanoscale thickness utilized in several applications such as membrane-based water treatment and adsorption [25-27]. Distribution of nano-clay in polymeric matrices can improve mechanical properties as well as the reduction of oxygen permeability which is among the factors affecting food preservation through generating tortuous paths in the polymeric film. Montmorillonite (MMT) is among the most common nano-clays which have been studied extensively as a nano-filler in various polymeric matrices, especially natural polymers [29, 30]. Based on the reported studies, addition of < 0.5 % MMT to gelatin leads to a significant improvement of mechanical and thermal properties [30, 31], while water vapor permeability and transparency are reduced [32]. Also, addition of MMT to chitosan-based films has led to the improvement of tensile strength and elongation at break and also water vapor permeability reduction [33-35].

In this study, effect of MMT addition on the

performance of gelatin-based nanocomposite films is investigated for food packaging application which is not reported in the literature to the best of our knowledge. Various MMT contents have been added to a gelatin-chitosan blend in order to investigate physical, mechanical and barrier properties. It is expected that MMT and chitosan improve gelatin properties to obtain nanocomposite films with a high strength and barrier properties as well as antimicrobial activity.

MATERIALS AND METHODS

Materials

Chitosan ($M_w=60000-80000$ g/gmol) is purchased from Acros. Na-MMT is supplied by Iranian nanomaterials Co. Gelatin, acetic acid and glycerol are also purchased from Merck Co. All materials are used as received without further purification. Deionized water is used for the preparation of solutions.

Methods

Preparation of gelatin and chitosan solutions

A 10 wt. /v % gelatin solution is prepared using deionized water as solvent by continuous stirring at 50 °C for 30 min [30]. Also, chitosan solution is prepared by addition of chitosan powder to 1 v/v % acetic acid and stirring at 40 °C for 6 h [33].

Preparation of gelatin-chitosan-MMT nanocomposite films

MMT is dispersed in deionized water (1 wt. /v %) using a magnetic stirrer and ultra-sonication. The as-prepared MMT suspension is added to a gelatin-chitosan solution in a dropwise manner. Finally, glycerol (30 wt.%) is added to the blend as a plasticizer. The as-prepared solution is poured in petri dish (10 cm in diameter) and maintained at 25 ± 2 °C. The dried samples were maintained under 5% relative humidity.

Characterization

In order to investigate the nanocomposite film structure and the distribution of MMT in the film matrix, X-ray diffraction (XRD) analysis was performed using a Bruker D8 device. Also, scanning electron microscopy (SEM, TESCAN VEGA3) was performed to show the distribution of MMT in the gelatin matrix. Thermogravimetric analysis (TGA) and water vapor permeability tests were also performed on the optimal film sample to analyze the thermal and water vapor barrier

properties, respectively.

Design of Experiments

Design of experiments was performed to observe the variation of response values by adjustment of input parameters. One common method for design of experiments is one-parameter-at-a-time strategy. In this method, interaction of parameters cannot be evaluated since one parameter is varied by keeping other parameters constant. However, factorial design can be utilized to assess the interaction of parameters while requiring a fewer number of experiments [36]. In this study, Design Expert 10 was implemented to design 19 experiments in order to evaluate the effect of gelatin, chitosan and MMT content on the nanocomposite film properties (Table 1).

RESULTS AND DISCUSSION

Characterization of Na-MMT

XRD analysis

Fig. 1 shows the XRD pattern of Na-MMT. The peaks observed at $2\theta = 7.61^\circ$ and 20.36° are attributed to 001 and 002 planes in MMT (Fig. 1).

Also, the peak observed at $2\theta = 13.76^\circ$ approves the existence of kaolinite (K) and the peaks at $2\theta = 27.26^\circ$ and 28.41° identify quartz (Q) in the sample [37]. Based on the XRD pattern and Bragg's equation (Eq. 1), the distance between 001 planes in MMT can be calculated as follows:

$$n\lambda = 2d \sin\theta \tag{1}$$

Where n is the refractive index, λ = wavelength of the XRD source (nm) and θ = Bragg's angle. Based on the XRD pattern, 7.61° is related to 001 plane from which the spacing is calculated as 1.16 nm.

FTIR spectrum

Fig. 2 shows the FTIR spectra for Na-MMT. The peaks observed at 3623 cm^{-1} and 3437 cm^{-1} wavenumbers are attributed to the stretching of -OH (free hydroxyl functional group) and also water. Also, the peak at 1618 cm^{-1} is related to water deformation and the peak at 1036 cm^{-1} corresponds to Si-O stretching vibration. Deformation of Al-Al-OH is observed at 915 cm^{-1} . The peak at 799 cm^{-1} , identifies the stretching vibration of Si-O due to the presence of quartz in

Table 1. Design of experiments.

No.	Gelatin (vol. %)	Chitosan (vol. %)	MMT (wt. %)	No.	Gelatin (vol. %)	Chitosan (vol. %)	MMT (wt. %)
1	85	15	3	11	90	10	7
2	90	10	0	12	95	5	5
3	80	20	3	13	100	0	3
4	90	10	3	14	90	10	3
5	100	0	3	15	100	0	0
6	85	15	1	16	95	5	1
7	80	20	3	17	90	10	0
8	90	10	7	18	100	0	7
9	80	20	0	19	85	15	5
10	80	20	7				

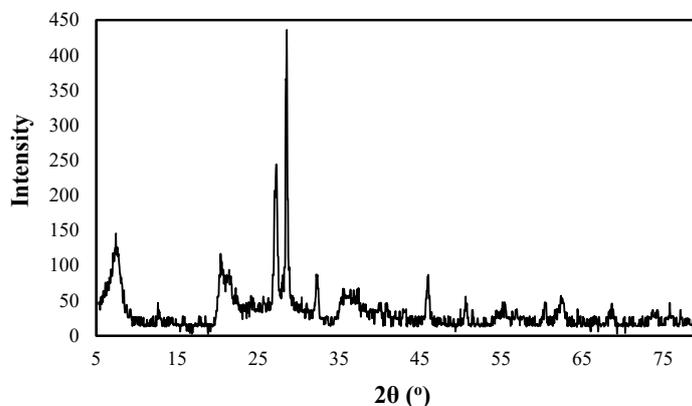


Fig. 1. XRD pattern for Na-MMT.



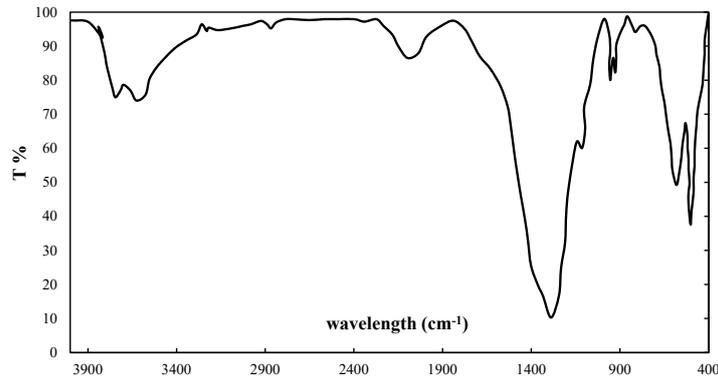


Fig. 2. FTIR spectrum of Na-MMT.

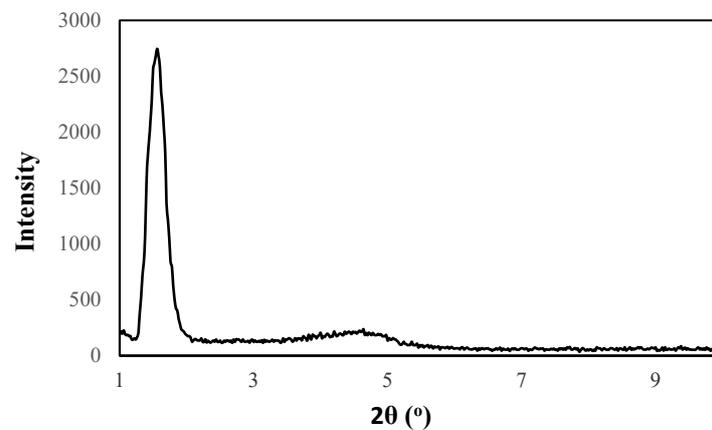


Fig. 3. XRD pattern of gelatin-chitosan-MMT nanocomposite film without ultra-sonication of Na-MMT for preparation of the nanocomposite film.

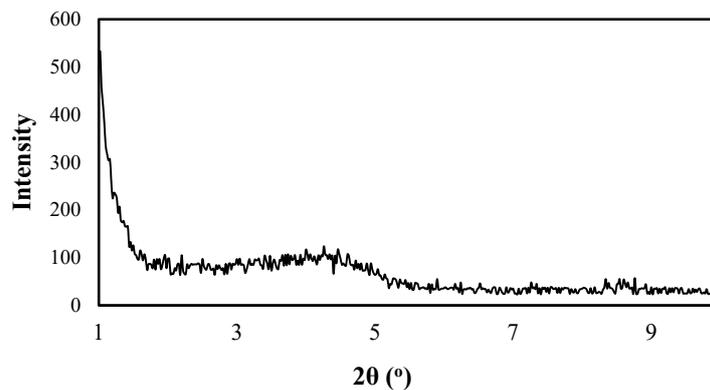


Fig. 4. XRD pattern of gelatin-chitosan-MMT nanocomposite film with ultra-sonication of Na-MMT for the preparation of nanocomposite film.

the compound. Also, Al-O-Si and Si-O-Si bands are observed at 531 and 473 cm^{-1} wavelength [38, 39].

Characterization of the nanocomposite film

XRD analysis

XRD analysis is performed to investigate the

crystalline structure of the nanocomposite film and also approving the enhancement of Na-MMT 001 plane spacing through the polymeric matrix (Fig. 3). The peak at $2\theta=1.56^\circ$, identifies the reflection of 001 plane in MMT. Bragg's equation shows the enhancement of plane spacing from 1.16 nm to

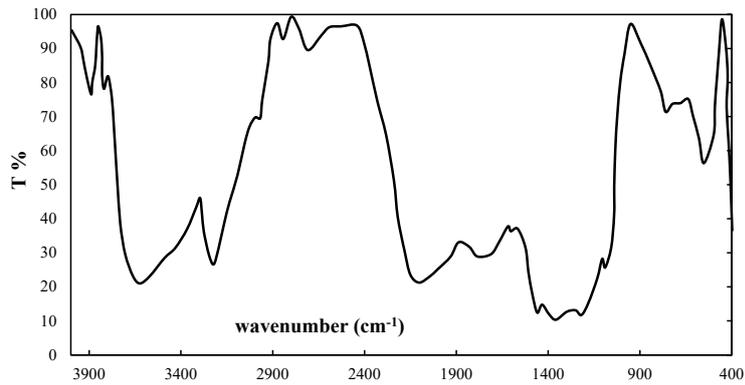


Fig. 5. FTIR spectrum of pure chitosan.

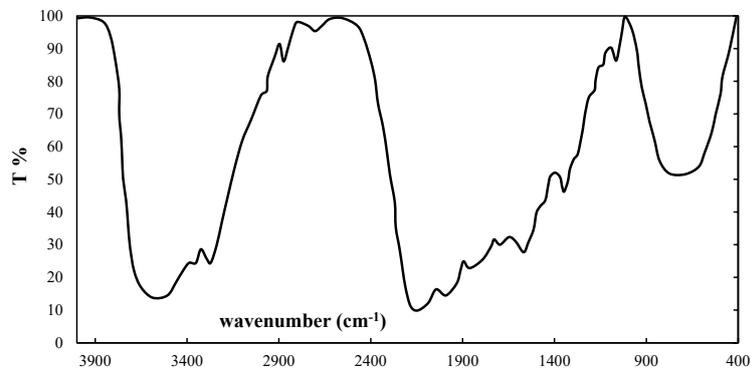


Fig. 6. FTIR spectrum of pure gelatin.

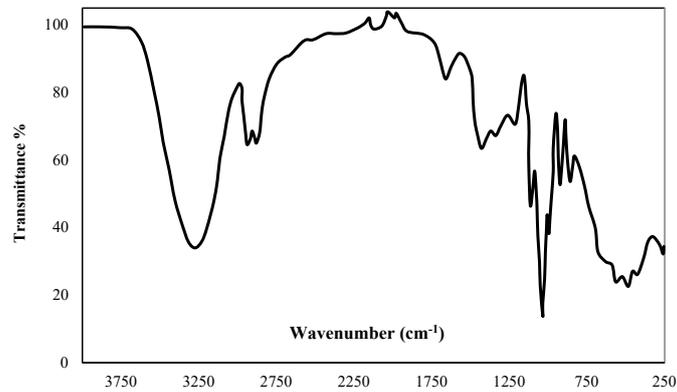


Fig. 7. FTIR spectrum of glycerol.

5.656 nm in the nanocomposite which shows the proper interaction of MMT with the polymeric blend. It is worth mentioning that nanocomposite preparation is performed without ultra-sonication of Na-MMT [40]. Ultra-sonication of Na-MMT results in the disappearance of the peak which corresponds to total exfoliation and dispersion in

the polymeric matrix (Fig. 4) [36].

FTIR Spectrum of the nanocomposite film

Molecular interactions in the nanocomposite film are characterized using FTIR spectra. Figs. 5 and 6 show the FTIR spectrum of pure gelatin and chitosan, respectively. The peaks in the 3000-3500

cm^{-1} range are attributed to the stretching bands of free hydroxyl and symmetrical and asymmetrical stretching bands of $-\text{NH}_2$ amine groups observed at 3448 cm^{-1} in the chitosan FTIR spectrum. The peak observed in the $2750\text{-}3000 \text{ cm}^{-1}$ range is attributed to the stretching of $-\text{CH}$, $-\text{CH}_2$ and $-\text{CH}_3$. Also, carbonyl bands ($\text{C}=\text{O-NHR}$) are observed in the $1400\text{-}1700 \text{ cm}^{-1}$ range which is observed at 1622 cm^{-1} in chitosan.

Fig. 6, shows the FTIR spectrum of pure gelatin. The stretching vibration observed at 3382 cm^{-1} is attributed to $-\text{OH}$ and $-\text{NH}_2$ and stretching vibrations at 2938 cm^{-1} , 1655 cm^{-1} , 1081 cm^{-1} and 1540 cm^{-1} wavenumbers are attributed to the presence of $-\text{CH}$, $-\text{C}=\text{O}$, $-\text{C-O}$ and $-\text{NH}$, respectively. Fig. 7 shows the FTIR spectrum of glycerol. The stretching vibration at 3268 cm^{-1} is due to $-\text{OH}$ free hydroxyl group and stretching vibrations of $-\text{CH}$ groups are observed at 2890 and 2934 cm^{-1} .

The band appeared in the $1200\text{-}1500 \text{ cm}^{-1}$ range is attributed to the bending vibration of $-\text{CH}$, $-\text{CH}_2$ and $-\text{CH}_3$ groups. Also, the peak at 1031 cm^{-1} is attributed to the stretching vibration of carbonyl group ($-\text{CO}$) [43].

Fig. 8 shows the FTIR spectra of the nanocomposite film. The stretching vibration related to $-\text{OH}$ is observed at 3260 cm^{-1} . Also, the bands appeared in the $1200\text{-}1500 \text{ cm}^{-1}$ range are attributed to the stretching vibration of $-\text{CH}$, $-\text{CH}_2$ and $-\text{CH}_3$ groups. Also, the bands in the $1200\text{-}1500 \text{ cm}^{-1}$ range are attributed to bending vibrations of $-\text{CH}$, $-\text{CH}_2$ and $-\text{CH}_3$ groups. Also, the peak observed at 1160 cm^{-1} is related to the stretching vibration of carbonyl functional group. It is worth mentioning that the enhancement of peak intensity in the $1500\text{-}1700 \text{ cm}^{-1}$ range (amino and carbonyl groups) approve the electrostatic interactions between gelatin and chitosan [41].

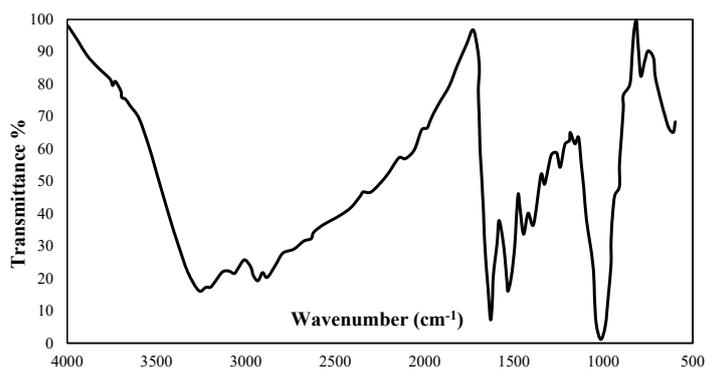


Fig. 8. FTIR spectrum of the nanocomposite film.

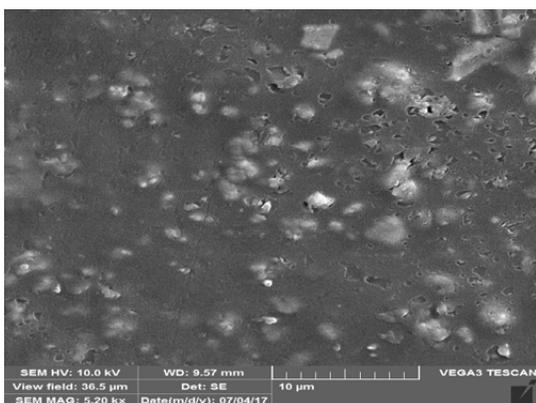


Fig. 9. SEM micrograph of the nanocomposite film without ultrasonication of MMT.

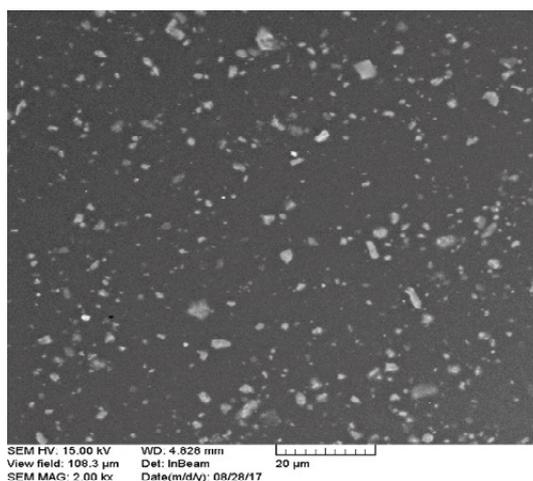


Fig. 10. SEM micrograph of the nanocomposite film with ultra-sonication of MMT before mixing with the polymeric blend.

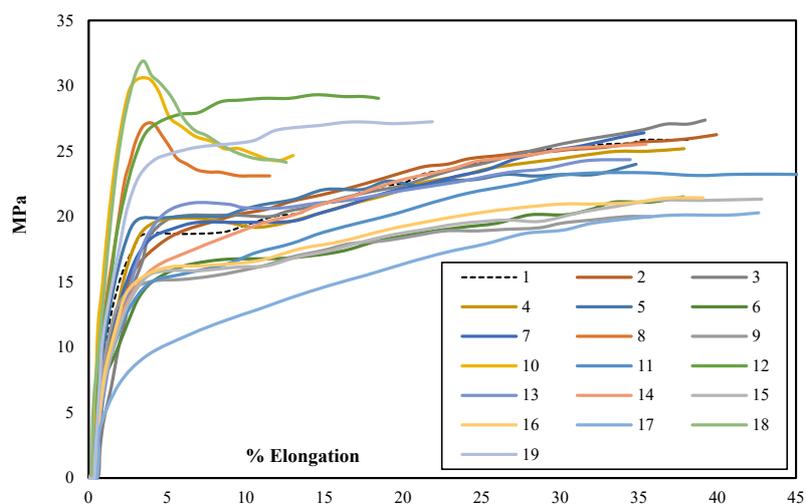


Fig. 11. Stress-Strain Curves for 19 film samples.

Table 2. ANOVA analysis for mechanical strength.

Source	Sum of squares	Mean square	F value	p-value
Model	1.069×10^{-5}	1.527×10^{-6}	105.98	< 0.0001
Linear mixture	3.439×10^{-7}	3.439×10^{-7}	23.87	0.0005
AC	3.103×10^{-7}	3.103×10^{-7}	21.54	0.0007
BC	2.021×10^{-7}	2.021×10^{-7}	14.03	0.0032
AC ²	1.138×10^{-6}	1.138×10^{-6}	78.96	< 0.0001
BC ²	3.068×10^{-6}	3.068×10^{-6}	212.91	< 0.0001
AC ³	1.485×10^{-7}	1.485×10^{-7}	10.31	0.0083
BC ³	4.276×10^{-8}	4.276×10^{-8}	2.97	0.1129
Residual	1.585×10^{-7}	1.441×10^{-8}		
Lack of Fit	9.916×10^{-8}	1.653×10^{-8}	1.39	0.3667
Corrected total	1.085×10^{-5}			

Also, the peaks appeared at 789 cm^{-1} and 1020 cm^{-1} show the stretching vibration of Si-O bond.

SEM analysis of the nanocomposite film

Fig. 9 shows the SEM micrograph of gelatin/chitosan/MMT nanocomposite film showing the distribution of MMT in the polymeric matrix at the intercalation state. It should be mentioned that MMT without ultra-sonication can enhance agglomeration which results in the reduction of mechanical properties in the nanocomposite film. Ultra-sonication of MMT before mixing with the polymeric blend leads to a homogeneous distribution of exfoliated clay nano-sheets (Fig. 10).

Mechanical Properties

Tensile test is the most common technique for evaluation of the mechanical properties of

polymeric films. Mechanical properties of 19 film samples (based on Table 1) are tested based on ASTM D638-02a standard using a GT-7010-D2E tensile strength device. For this purpose, Dumbbell-like portions of films were prepared and tested in the tensile strength device with a 10 mm/min velocity to evaluate the strength at break (TS) and elongation at break (EB) from the stress-strain curves (Fig. 11).

Tensile strength at break

Table 2 summarizes the results of designed experiments for mechanical strength. ANOVA analysis shows the significance of each parameter and the interaction of parameters on response values. P-value is smaller than 0.05 which confirms the model significance. Also, investigating the effect of MMT content on tensile strength, it can be inferred that tensile strength is enhanced up

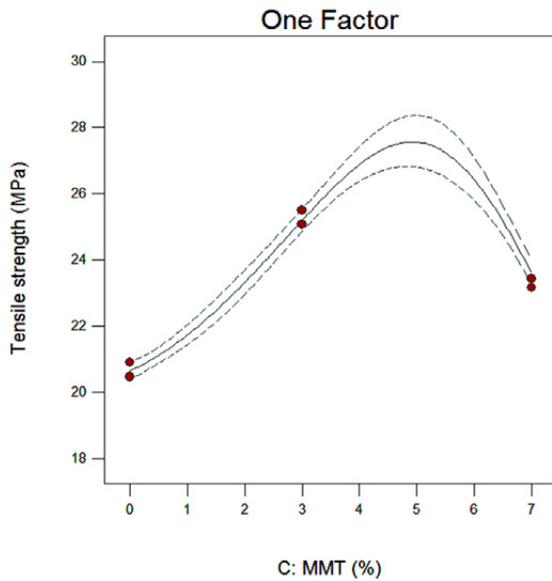


Fig. 12. Effect of MMT content on the tensile strength of the nanocomposite film.

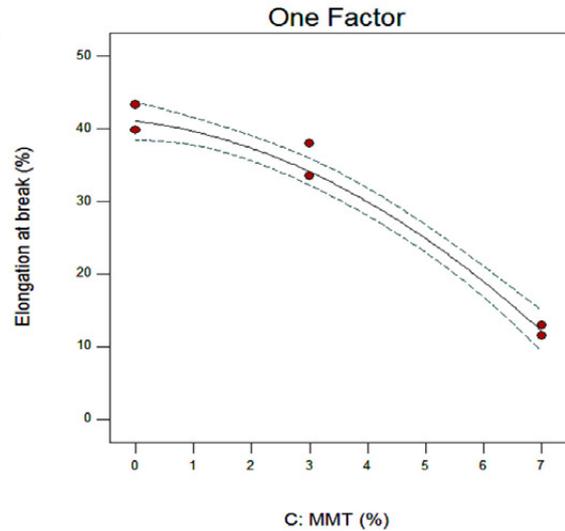


Fig. 13. Effect of MMT content on the elongation at break of the nanocomposite film.

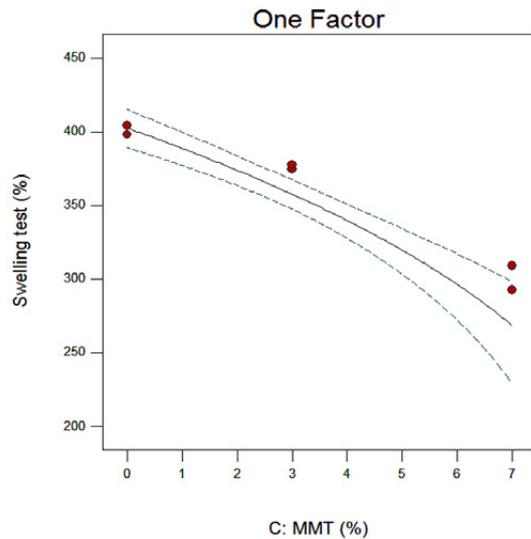


Fig. 14. Effect of MMT content on the swelling behavior of the nanocomposite film.

to 5 wt. % MMT content after which strength decline is observed, which can be attributed to the agglomeration of MMT in the polymeric matrix above the 5 wt. % value (Fig. 12). Also, presence of chitosan in the nanocomposite film results in a slight improvement in the mechanical strength.

Elongation at break (EB)

Elongation at break shows the flexibility

of the film samples before final breaking [42]. Investigating the results based on ANOVA results (Table 2), it can be mentioned that the most influential parameter on mechanical strength is the gelatin-MMT interaction. EB is reduced significantly by addition of MMT into the gelatin matrix so that 7 wt. % addition of MMT results in 75% reduction of EB in the nanocomposite film. Also, chitosan has almost no effect on EB (Fig. 13).

Swelling behavior

Generally, natural polymers are swollen in the vicinity of moisture which results in the exposure of the packaged food to water and upcoming deterioration. To this end, the swelling behavior of nanocomposite samples was examined through the technique introduced by Farahnaki *et al.* [41]. Based on the experiments, MMT addition from 1 to 7 % results in the reduction of swelling in the nanocomposite films from 400 % to ~250 % mostly due to the intervention of MMT sheets with free movement of the polymeric chains. Also, chitosan leads to the reduction of swelling tendency (Fig. 14).

Transparency

Transparency of films and coatings is of great significance in the packaging industry due to the inclination of customers to observe the food material through the packaging film [3]. However,

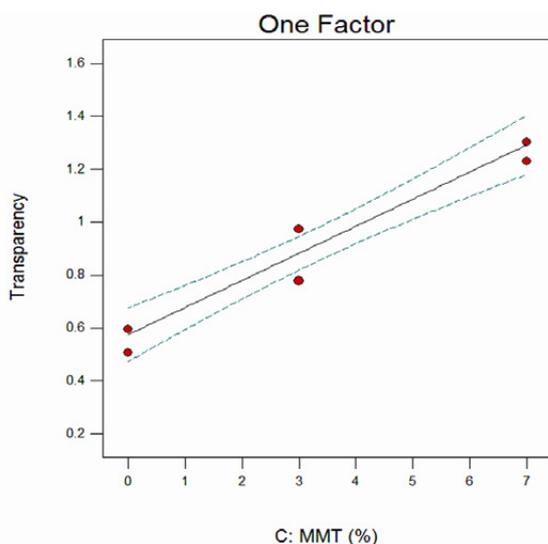


Fig. 15. Effect of MMT content on transparency of the nano-composite films.

there is a compromise between the nano-filler content and film transparency which can be characterized by UV absorption. As a result, UV absorption of nanocomposite samples at 600 nm is evaluated using a UV-vis spectrophotometer (JASCO V-730) [43] and summarized in Table 2. Based on the results, transparency is significantly reduced at high MMT loadings. So, a compromise should be made between transparency and mechanical strength. Also, chitosan leads to the reduction of transparency in the nanocomposite film at similar MMT contents in comparison with pure gelatin. However, this trend is reversed at higher gelatin: chitosan ratios (Fig. 15).

Water contact angle

In order to investigate the samples behavior in the vicinity of moisture and their attachment to food which is problematic in food packaging, water contact angle test was performed (Table 2). For this purpose, the contact angle of a 10 μ L water droplet on the samples surface was observed and measured using a digital microscope (USB, 500X) [44]. Based on the results, MMT addition into the polymeric matrix at a low weight percent can enhance water contact angle of the nanocomposite films to a small extent. However, higher MMT contents can reduce the water contact angle significantly (Fig. 16) which can be attributed to the hydrophilic nature of MMT [34]. Also, chitosan can result in the reduction of

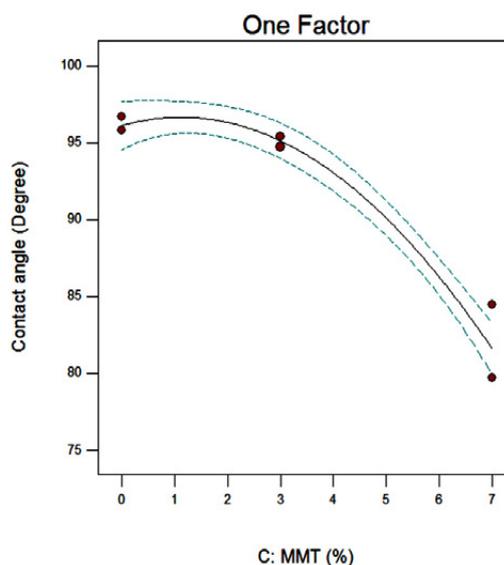


Fig. 16. Effect of MMT on water contact angle for nanocomposite films.

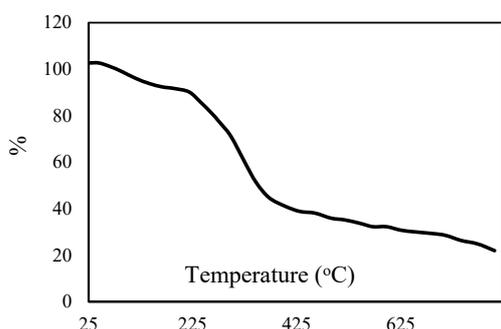
water contact angle which is due to the higher hydrophilicity of chitosan compared to gelatin.

OPTIMIZATION AND COMPARISON

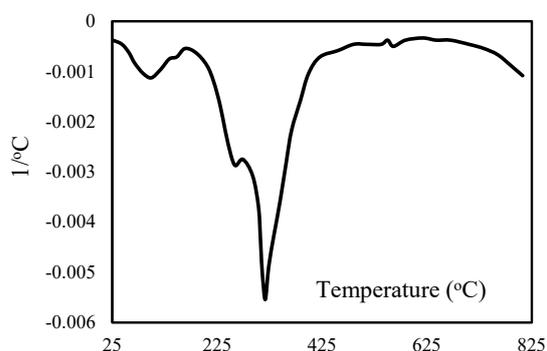
Optimization targets were selected as the highest tensile strength, elongation at break and water contact angle. Also, the lowest swelling and turbidity values are expected. The sample containing 80 wt. % gelatin, 20 wt. % chitosan and 3.04 wt. % MMT was selected as the optimal sample. For this sample, tensile strength=26.531 MPa, EB=36.491%, water contact angle=94.94° and swelling =331.495 % was predicted by Design Expert software. Also, thermal stability and water vapor permeability tests were performed on the optimized samples which are reported in subsections 4.1 and 4.2.

Thermal stability

TGA test was performed on the optimal sample to investigate the film thermal stability (Fig. 17). It is predicted that nano-clay can enhance the thermal stability of nanocomposite film which is due to the thermal resistance of nano-clay and the improved interaction with the polymeric matrix [45, 46]. Based on Fig. 17 (a, b), the initial weight loss in a smaller than 100 °C domain can be due to moisture loss and also loss of volatile compounds adsorbed in the nanocomposite film. The same result is observed around 170 °C which is due to the reduction



a)



b)

Fig. 17(a, b). TGA analysis for the optimal nanocomposite film.

of absorbed and bound water. The second reduction trend is attributed to the destruction of gelatin and chitosan which is initiated around 290 °C up to 440 °C [47]. The 3rd reduction (T > 550 °C) is also due to the de-hydroxylation peak in nano-clay.

Water Vapor Permeability (WVP)

Water vapor permeability test is performed on films with chitosan/gelatin ratios of 80:20 and

No.	[Citosan:Gelatin]	MMT %	WVP (g.mm/h.cm ² .Pa)
1	80:20	0	9.14×10 ⁻⁸
2	80:20	3	8.14×10 ⁻⁸
3	80:20	5	8.71×10 ⁻⁸
4	80:20	7	1.06×10 ⁻⁷
5	90:10	0	9.49×10 ⁻⁸
6	90:10	3	9.29×10 ⁻⁸
7	90:10	5	9.59×10 ⁻⁸
8	90:10	7	9.57×10 ⁻⁸

Table 3. Water vapor permeability at various MMT contents.

90:10 for various MMT contents of 0, 3, 5 and 7 %. The results are summarized in Table 3. Based on the results, MMT addition at small contents results in the reduction of WVP while enhanced at higher contents due to agglomeration. Also, chitosan results in the reduction of WVP in the nanocomposite film.

COMPARISON OF THE NANOCOMPOSITE WITH POLYETHYLENE FOR FOOD PACKAGING

The fabricated nanocomposite films with a chitosan to gelatin ratio of 8:20 and 3 wt. % of MMT was used for pomegranate seeds packaging. Also, polyethylene (PE) film samples were used for comparison.

Figs. 18 (a & b) and Figs. 19 (a & b), show the pomegranate seeds packaged using PE films and the nanocomposite film, respectively. As it is obvious from Fig. 18, after 6 days fungal growth is observed on the preserved pomegranate seeds under laboratory conditions. Simultaneously, pomegranate seeds were preserved in the nanocomposite film under similar conditions. Based on Fig. 19, there is no clue of fungal growth



Fig. 18. a) Pomegranate seeds packaged with PE film initially and, b) after 6 days.

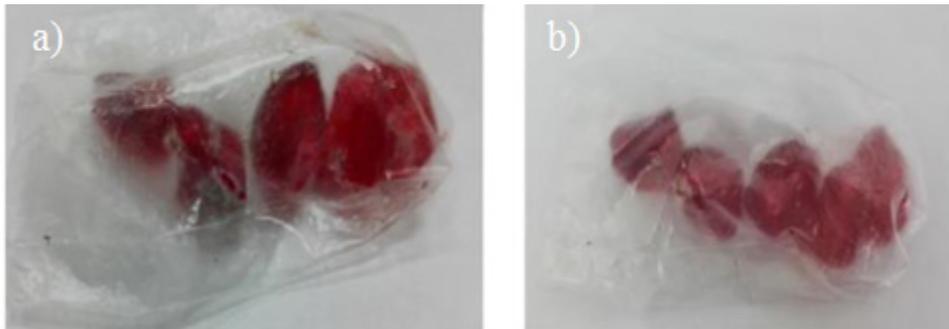


Fig. 19. a) Pomegranate seeds packaged with the prepared nanocomposite film initially and, b) after 6 days.

on pomegranate seeds packaged using the prepared nanocomposite film.

CONCLUSIONS

In this study, MMT is added to the gelatin-chitosan composite to improve the mechanical properties and water vapor permeability. The following results can be mentioned:

- (1) Addition of MMT up to 5 % results in the enhancement of tensile strength. After 5 %, agglomeration leads to significant reduction of tensile strength. Also, EB is reduced at all concentrations.
- (2) Addition of MMT to the composite matrix leads to the reduction of swelling and transparency.
- (3) Addition of MMT results in the reduction of contact angle.
- (4) Up to 3%, MMT addition leads to the reduction of WVP, after which WVP is enhanced significantly due to agglomeration of MMT.

CONFLICT OF INTERESTS

There is no conflict of interest.

REFERENCES

- [1] Popovic S. Z., Lazic V. L., Hromiš N. M., Šuput D. Z., Bulut S. N., (2018), Biopolymer packaging materials for food shelf-life prolongation. In *Biopolymers for Food Design, Chapter 8*; Grumezescu, A.M., Holban, A.M., Eds.; Academic Press: New York, NY, USA, 223–277.
- [2] Silvestre C., Duraccio D., Cimmino S., (2011), Food packaging based on polymer nanomaterials. *Prog. Polym. Sci.* 36: 1766–1782.
- [3] The New Plastics Economy: Rethinking the future of plastics (2016), World economic forum.
- [4] Medina-Jaramillo C., Ochoa-Yepes O., Bernal C., Famá L., (2017), Active and smart biodegradable packaging based on starch and natural extracts. *Carbohydr. Polym.* 176: 187–194.
- [5] Stark N. M., (2016), Opportunities for cellulose nanomaterials in packaging films: A review and future trends. *J. Renew. Mater.* 4: 313-326.
- [6] Syahida N., Fitri I., Zuriyati A., Hanani N., (2020), Effects of palm wax on the physical, mechanical and water barrier properties of fish gelatin films for food packaging application. *Food Packag. Shelf Life.* 23: 100437-100442.
- [7] Yang W., Owczarek J. S., Fortunati E., Kozanecki M., Mazzaglia A., Balestra G. M., Kenny J. M., Torre L., Puglia D., (2016), Antioxidant and antibacterial lignin nanoparticles in polyvinylalcohol/chitosan films for active packaging. *Indust. Crops Prod.* 94: 800–811.
- [8] Kumar S., Boro J. C., Ray D., Mukherjee A., Dutta J., (2019), Bio-nanocomposite films of agar incorporated with ZnO nanoparticles as an active packaging material for shelf life extension of green grape. *Heliyon.* 5: e01867.
- [9] Luchian-Lupu A., Zaharescu T., Lungulescu E., Răpă M., Iovu H., (2020), Availability of PLA/SIS blends for packaging and medical applications. *Radiation Phys. Chem.* 172: 108696-108701.
- [10] Benhacine F., Ouargli A., Hadj-Hamou A. S., (2019), Preparation and characterization of novel food packaging materials based on biodegradable PCL/Ag-kaolinite nanocomposites with controlled release properties. *Polymer-Plastics Tech. Mater.* 58: 328-340.
- [11] Fabra M. J., López-Rubio A., Ambrosio-Martín J., Lagaron J. M., (2016), Improving the barrier properties of thermoplastic corn starch-based films containing bacterial cellulose nano-whiskers by means of PHA electro-spun coatings of interest in food packaging. *Food Hydrocol.* 61: 261-268.
- [12] Noorbakhsh-Soltani M., Zerafat M. M., Sabbaghi S., (2018), A Comparative study of gelatin and starch-based natural nanocomposite films modified by nano-cellulose and chitosan for food packaging applications. *J. Carbohydr. Polym.* 189: 48-55.
- [13] Bae H. J., Park H. J., Hong S. I., Byun Y. J., Darby D. O., Kimmel R. M., Whiteside W. S., (2009), Effect of clay content, homogenization RPM, pH, and ultra-sonication on mechanical and barrier properties of fish gelatin/montmorillonite nanocomposite films. *LWT - Food Sci. Tech.* 42: 1179–1186.
- [14] Mao J., Kondu S., Ji H. F., McShane M. J., (2006), Study of the near-neutral pH-Sensitivity of chitosan/gelatin hydrogels by turbidimetry and microcantilever deflection. *Biotech. Bioeng.* 95: 333-341.
- [15] Jayakumar R., Nwe N., Tokura S., Tamura H., (2007), Sulfated chitin and chitosan as novel biomaterials. *Int. J.*

- Biolog. Macromol.* 40: 175-181.
- [16] Rinaudo M., (2008), Main properties and current applications of some polysaccharides as biomaterials. *Polymer Int.* 57: 397-430.
- [17] Appendini P., Hotchkiss J. H., (2002), Review of antimicrobial food packaging. *Innov. Food Sci. Emerg. Tech.* 3: 113-126.
- [18] Bandyopadhyay J., Ray S. S., (2019), Are nanoclay-containing polymer composites safe for food packaging applications? An overview. *J. Appl. Polym. Sci.* 136: 47214-47219.
- [19] Samandari S. S., (2014), Elastic modulus measurement of polymer matrix nano-composites reinforced by platelet nano-clays. *Int. J. Nano Dimens.* 5: 273-278.
- [20] Heidarbeigi J., Borghei A. M., Afshari H., (2019), The mechanical and thermal properties of PE/CNC nanocomposite. *Int. J. Nano Dimens.* 10: 209-216.
- [21] Kuorwel K. K., Cran M. J., Orbell J. D., Buddhadasa S., Bigger S. W., (2015), Review of mechanical properties, migration, and potential applications in active food packaging systems containing nano-clays and nano-silver. *Compr. Rev. Food Sci. Food Saf.* 14: 411-430.
- [22] Alidadykhah M., Peyman H., Roshanfekar H., (2021), Application of a new polymer AgCl nanoparticles coated polyethylene terephthalat [PET] as adsorbent for removal and electrochemical determination of methylene blue dye. *Chem. Methodol.* 5: 96-106.
- [23] Abdia R., Ghorbani-HasanSaraeia A., Naghizadeh Raeisia Sh., Karimi F., (2020), A gallic acid food electrochemical sensor based on amplification of paste electrode by Cdo/CNTs nanocomposite and ionic liquid. *J. Med. Chem. Sci.* 3: 338-344.
- [24] Choudalakis G., Gotsis A., (2009), Permeability of polymer/clay nanocomposites: A review. *Eur. Polym. J.* 45: 967-984.
- [25] Foorginezhad S., Zerafat M. M., (2019), Preparation of low cost ceramic membranes using persian natural clay and application for dye clarification. *Desal. Water Treat.* 145: 378-392.
- [26] Foorginezhad S., Zerafat M. M., (2017), Microfiltration of cationic dyes using nano-clay membranes. *Ceram. Int.* 43: 15146-15159.
- [27] Asadi F., Zerafat M. M., (2016), Adsorption of toluene from aqueous solutions using clay-based nanostructures. *Sep. Sci. Tech.* 51: 569-574.
- [28] Saadat F., Zerafat M. M., Foorginezhad S., (2020), Adsorption of copper ions from aqueous media using montmorillonite- Al_2O_3 nano-adsorbent incorporated with Fe_3O_4 for facile separation. *Korean J. Chem. Eng.* 37: 2273-2286.
- [29] Tang X. Z., Kumar P., Alavi S., Sandeep K. P., (2012), Recent advances in biopolymers and biopolymer-based nanocomposites for food packaging materials. *Crit. Rev. Food Sci. Nutrition.* 52: 426-442.
- [30] Mu Ch., Li X., Zhao Y., Zhang H., Wang L., Li D., (2012), Freezing/Thawing effects on the exfoliation of montmorillonite in gelatin-based bio-nanocomposite. *J. Appl. Polym. Sci.* 128: 3141-348.
- [31] Rao Y. Q., (2007), Gelatin-clay nanocomposites of improved properties. *Polymer.* 48: 5369-5375.
- [32] Flaker Ch. H. C., Lourenço R. V., Bittante A. M. Q. B., Sobral P. J. A., (2015), Gelatin-based nanocomposite films: A study on montmorillonite dispersion methods and concentration. *J. Food Eng.* 167: 65-70.
- [33] Abdollahi M., Rezaei M., Farzi Gh., (2012), A novel active bio-nanocomposite film incorporating rosemary essential oil and nano-clay into chitosan. *J. Food Eng.* 111: 343-350.
- [34] Giannakas A., Grigoriadi K., Leontiou A., Barkoula N. M., Ladavos A., (2014), Preparation, characterization, mechanical and barrier properties investigation of chitosan-clay nanocomposites. *Carbohydr. Polym.* 108: 103-111.
- [35] Collins L. M., Dziak J. J., Li R., (2009), Design of experiments with multiple independent variables: a resource management perspective on complete and reduced factorial designs. *Psychol. Methods.* 14: 202-24.
- [36] Müller C. M., Laurindo J. B., Yamashita F., (2011), Effect of nano-clay incorporation method on mechanical and water vapor barrier properties of starch-based films. *Ind. Crops Prod.* 33: 605-610.
- [37] Esteghlal S., Niakosari M., Hosseini M. H., Mesbahi Gh. R., Yousefi Gh. H., (2016), Gelatin-hydroxy-propyl methylcellulose water-in-water emulsions as a new bio-based packaging material. *Int. J. Biol. Macromol.* 86: 242-249.
- [38] Adikary S., Ashokline M., Nirojan K., (2015), Characterization of montmorillonite clay from naturally occurring clay deposits in Murunkan Area. *Proceedings of 8th International Research Conference*, KDU.
- [39] Chen G., Chen Sh., Qi Z., (2001), FTIR spectra, thermal properties and dispersibility of a polystyrene/montmorillonite nanocomposite. *Macromol. Chem. Phys.* 202: 1189-1193.
- [40] Zheng J. P., Wang Ch. Z., Wang X. X., Wang H. Y., Zhuang H., Yao K. D., (2007), Preparation of biomimetic three-dimensional gelatin/ montmorillonite-chitosan scaffold for tissue engineering. *React. Funct. Polym.* 67: 780-788.
- [41] Pereda M., Ponce A. G., Marcovich N. E., Ruseckaite R. A., Martucci J. F., (2011), Chitosan-gelatin composites and bi-layer films with potential antimicrobial activity. *Food Hydrocol.* 25: 1372-1381.
- [42] Rubilar J. F., Zúñiga R. N., Osorio F., Pedreschi F., (2015), Physical properties of emulsion-based hydroxyl-propyl methylcellulose/whey protein isolate (HPMC/WPI) edible films. *Carbohydr. Polym.* 123: 27-38.
- [43] Kanmani P., Rhim J. W., (2014), Physicochemical properties of gelatin/silver nanoparticle antimicrobial composite films. *Food Chem.* 148: 162-169.
- [44] Farahnaky A., Dadfar M. M., Shahbazi M., (2014), Physical and mechanical properties of gelatin-clay nanocomposite. *J. Food Eng.* 122: 78-83.
- [45] Zheng J. P., Li P., Ma Y. L., Yao K. D., (2002), Gelatin/montmorillonite hybrid nanocomposite. I. Preparation and Properties. *J. Appl. Polym. Sci.* 86: 1189-1194.
- [46] Martucci J. F., Vázquez A., Ruseckaite R. A., (2007), Nanocomposites based on gelatin and Montmorillonite, morphological and thermal studies. *J. Therm. Anal. Calorim.* 89: 117-122.
- [47] Leceta I., Guerrero P., Ibarburu I., Dueñas M. T., de la Caba K., (2013), Characterization and antimicrobial analysis of chitosan-based films. *J. Food Eng.* 116: 889-899.