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Improvement Physical Properties of Pullulan-Whey Protein Biocomposite Films with Nanoclay

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ABSTRACT

In the current study, whey protein- pullulan- clay nanocomposite films are prepared by casting method. The effect of nanoclay at three concentrations (1%, 3% and 5%) on physical properties such as moisture content, solubility in water, water vapor permeability and transparency of whey protein- pullulan composite films investigated. The results show that the effect of nanoparticles on composite depends on kind of nanoparticle and level of incorporation. Nanoclay particles changed solubility, water absorption and moisture content of the films but did not influence on transparency.

Keyword: Edible films; Whey protein; Pullulan; Nano clay; Biodegradable; Biocomposite.

1. INTRODUCTION

Approximately 125mt plastic is produced in the world yearly. 41% of plastic consumption is related to packaging industry of which food packaging accounts for 47%. Pollution resulted from packaging materials produced by oil derivatives as well as problems resulted from different methods of fumigation (such as burning, burning and recycling) have made researchers to find appropriate substitutes for these packaging materials. One of proposed solutions is to use from edible and biodegradable films as a substitute for petroleum undegradable polymers [11]. In this research pullulan and whey protein were used as biodegradable biopolymer. Pullulan is an extracellular microbial polysaccharide that produces by Aureaobasidium pullulans. Pullulan films are biodegradable, transparent, highly impermeable to oil and impermeable to oxygen [11]. Whey protein is the byproduct of cheese industry which is left after case in has been curd at 20°C and pH= 4.64.

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Use of whey protein for film production seems desirable because of high nutritional value of whey, optimal use of cheese making wastes and decrease of environmental problems [7]. As it said biopolymers have advantages than synthetic polymers among which biodegradability and renewability are the most important. However mechanical and barrier properties are two basic drawbacks of biopolymers which limit industrial use of these materials for packaging. To solve this problem use of nanoparticles has been proposed since it improves biopolymer efficiency as well as mechanical, thermal and barrier properties. Therefore a nanoparticle has an important role at improvement of biopolymer properties and can be used for food packaging [16, 20]. Capability of nanoclay at diffusing as separate layers, changing surface characteristics of these substances, compatibility with different kinds of polymers and biopolymers. Lower price and easier availability are among causes that have enhanced use of nanoclay for producing biopolymer nanocomposites [22].

Various natural biopolymer, including carbohydrate such as cellulose [14] starch [4] and chitosan [17] and protein such as soy protein [9, 10] gelatin [15] and whey protein [19] have been tested to exploit the property enhancement through the formation of nanocomposites. The main purpose of this study is improve barrier properties of WPI with PUL and nanoclay and investigated physical properties of these films.

2. MATERIALS AND METHODS

2.1. Material

WPI was purchased from (Arla food ingredient, Denmark), pullulan was provided from Hayashibara (Hayashibara Co LTD, Japan) and nanoclay was supplied by TECNAN (Tecnologia Navarra de Nanoproductos S.L, Spain). The analytical grade chemicals including sodium chloride (NaCl), calcium chloride ($CaCl_2$) and calcium nitrite ($Ca(NO_2)_2$) were purchased from Merck (Merck Co, Germany).

2.2. Method

2.2.1. Film preparation

5 g WPI dissolved in 100 mL distilled water and heated at 90°C for 30 min and stirred with magnetic stirrer. Then 5 g pullulan dissolved in 100 mL distilled water and mixed with WPI solution (1:1) w/w after that 30% w/v glycerol added into the solution. Three clay suspension prepared by ultrasonic bath (Elma, s 60 h, Germany) at ambient temperature for 1 h. So that the concentration of clay in WPI/PUL filmogenic solution was 1%, 3% and 5% wt (dray base). Finally after drying the solution at 25°C, the films peeled out and conditioned at 25°C and relative humidity 50% for 48 h before all tests.

2.2.2. Moisture content

Moisture content (MC) of film specimens were determined by weight of sample before (m1) and after (m2) oven drying (Shimaz co., Heraeus, Germany) at 105°C (Eq. 1).

$$MC = \frac{m_1 - m_2}{m_2} \times 100 \quad (1)$$

2.2.3. Moisture absorption

The film specimens have conditioned by calcium sulphate at RH= 0% for 24 h then weight (m1) and placed them in desiccator containing saturated solution of calcium nitrite at -25°C in order to achieve 55% relative humidity. After equilibrium state the samples weighed (m2) and Moisture absorption (MA) calculated by Eq. 2.

$$MA = \frac{m_1 - m_2}{m_2} \times 100 \quad (2)$$

2.2.4. Water vapor permeability

Water vapor permeability was measured according to ASTM E96 [1]. The films were fixed on the top of glass cup containing $CaCl_2$ and sealed with melted paraffin then the cups placed in a desiccator contain saturated NaCl solution and the cups weighted every hour for a period of 24 hour. The

slope (S) of time-weight was calculated by linear regression model ($R^2 \geq 0.986$). WVTR and WVP were calculated by Eq. 3 and Eq. 4.

$$WVTR = \frac{S}{A} \quad (3)$$

$$WVP = \frac{WVTR \times X}{\Delta P} \quad (4)$$

Where A is the effective film area (m^2), x is the average film thickness (m) and ΔP is the driving force (1753.55 Pa).

2.2.5. Solubility in water

The film specimens were dried at 105°C, and weighed (mL) then dried samples immersed into 50 ml of distilled water for 6 h. The remained films were dried at 105°C and weighed (m2). Solubility of the film (SW) in water calculates by Eq. 5.

$$SW = \frac{m_1 - m_2}{m_2} \times 100 \quad (5)$$

2.2.6. Transparency

Transparency of the films was determined by (Testo 540 pocket sized lux meters, UK).

2.2.7. Scanning electron microscopy

The morphology of the surface and the cross-section of films were observed by field emission scanning electron microscopy (FE-SEM) KYKY-EM3200 (KYKY, China) with the accelerating beam at a voltage 5 kV. The film specimens were sputtered with a thin layer of gold using a KYKY-SBC-12 sputter coater (KYKY, China).

2.2.8. Statistical analysis

Statistics on a completely randomized design were performed with the analysis of variance (ANOVA) procedure using SPSS software (Version 20; SPSS Inc., USA). Duncan's multiple range tests were used to compare the difference among mean values of film specimen's properties at the level of 0.05.

3. RESULTS

3.1. Moisture content

Moisture content of WPI-PUL and nanocomposite films observed in Figure 1. Moisture content of WPI-PUL film is 16.93% and decreased slowly and reaches to 14.85%. Lowest moisture content is 12.99% at 3% clay concentration increasing nanoclay content up to 5% increased moisture content. However this enhancement was not si gnificantly difference.

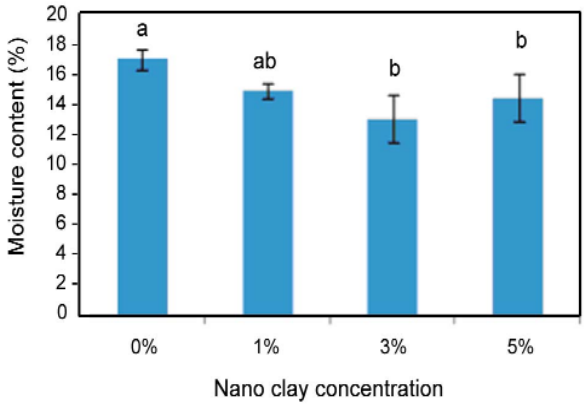


Figure 1: *Effect of clay content on moisture content of WPI-PUL film.*

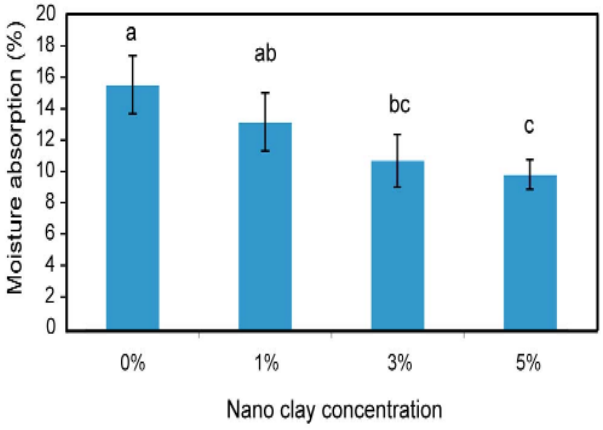


Figure 2: *Effect of clay content on moisture absorption of WPI-PUL film.*

3.2. Moisture absorption

One of important problems at using biopolymers is their tendency to absorb water. Results related to

amount of water absorption by WPI-PUL film in Figure 2 indicated that moisture absorption of WPI-PUL is 15.48% and reduced significantly by addition of nanoclay so that it reaches to 9.81% at concentration of 3% clay concentration.

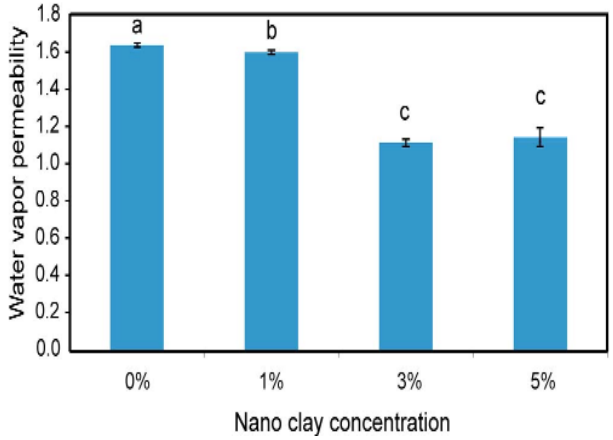


Figure 3: *Effect of clay content on water vapor permeability of WPI-PUL film.*

3.3. Water vapor permeability

As a result that has shown in Figure 3, Water vapor permeability of WPI-PUL film is 1.62×10^{-10} g m^{-1} s^{-1} pa^{-1} that indicates weak barrier properties of this biopolymer. By addition of 1% and 3% nanoclay water vapor permeability was reduced to 1.59×10^{-10} $gm^{-1}s^{-1}pa^{-1}$ and 1.1×10^{10} $gm^{-1}s^{-1}pa^{-1}$ respectively.

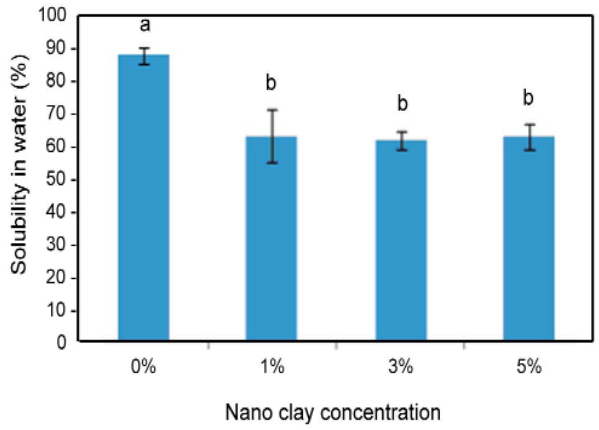


Figure 4: *Effect of clay content on solubility in water of WPI-PUL film.*

3.4. Solubility in water

Figure 4 Shows solubility in water of WPI-PUL and its nanocomposite films. Solubility in water related to the clay concentration. Solubility in water of WPI-PUL film is 87.46% and decreased significantly to 62.84% at 1% clay concentration. Lowest solubility is 61.35% and observed at 3% clay concentration.

3.5. Transparency

Figure 5 Shows that WPI-PUL film have high transparency 92% and decreased slowly by additional of clay concentration but transparency didn't change significantly for all samples.

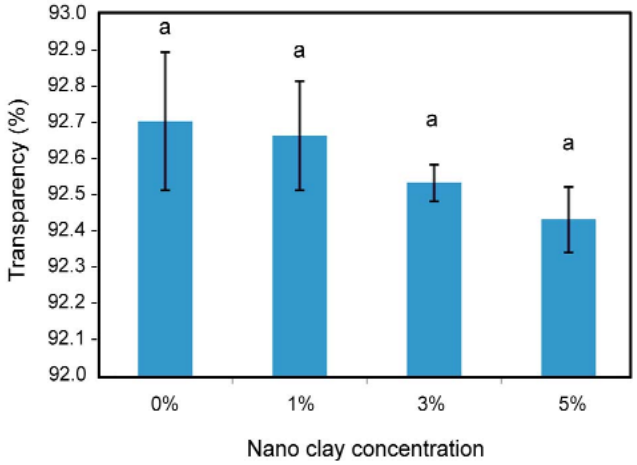
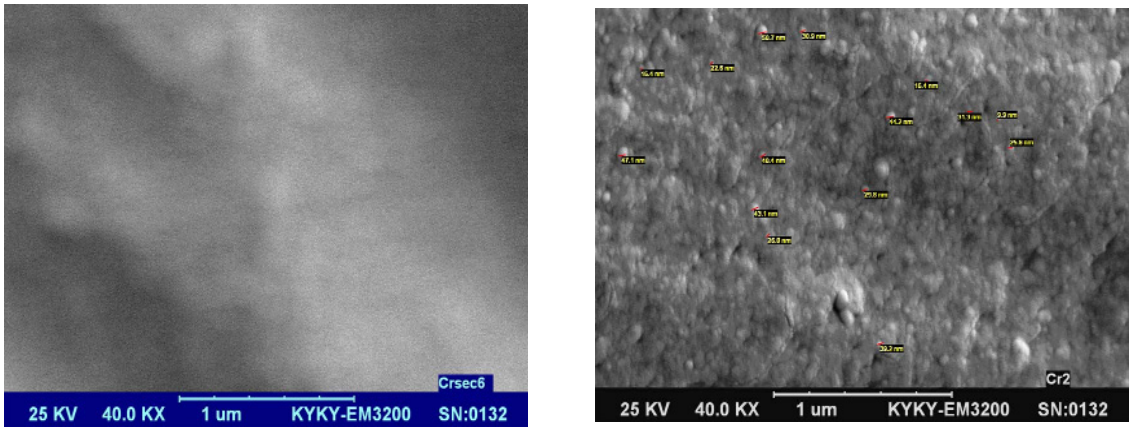


Figure 5: *Effect of clay content on transparency of WPI-PUL film.*



(a) (b)

Figure 6: *Cross section of WPI-PUL and WPI-PUL - 3% nanoclay composite film.*

3.6. SEM micrograph

Figure 6 exhibited the cross section of WPI-PUL and nanocomposite film with 3% clay concentration. Cross section of WPI-PUL film is smooth and homogenous. At 3% clay concentration white particle appear on the cross section of the film and the size of nano particle was determined.

4. DISCUSSION

4.1. Moisture content

As show in Figure 1, Nanoclay decreased moisture content of nanocomposite films. It may be due to good dispersion of nanoclay particle in polymer matrix and good interaction between polymer and nanoclay particles. These interactions reduce the occupied void volume of polymer matrix by water molecules [7]. Increasing nanoclay content up to 5% increased moisture content. However this enhancement was not significantly difference but presumably it is due to declining homogeneity of nanoclay particles in polymer matrix. Increasing nanoclay content up to 5% increased moisture content. However this enhancement was not significantly different but presumably it is due to declining homogeneity of nanoclay particles in polymer matrix.

4.2. Moisture absorption

One of important problems at using biopolymers is their tendency to absorb water. As shown in Figure 2, nanoclay decreased water absorption of biopolymer. It may be attributed to creation of hydrogen bond between hydroxyl group of polymer and oxygen atoms in nanoclay. The occupation of hydroxyl group of polymer with nanoclay decreases absorption of water by polymer matrix [6]. Similar results have been reported in CMC-starch composite films [2].

4.3. Water vapor permeability

One of the most important uses of food packaging is reduce moisture between food and atmosphere and water vapour permeability should be at minimum. Reduced water vapour permeability of nanoclay composites is due to presence of silicate impervious layers dispersed into polymeric matrix creating tortuous path way for molecules. Therefore these molecules should go around the layers to pass the film which results in enhancement of time and way length thus reduction in permeability [18]. In addition hydrophilic properties of nanoclay and its compatibility with hydrophilic biopolymer lead to improvement of water vapour permeability. Reduced water vapour permeability due to addition of nanoclay in other biopolymers such as chitosan

[17] and thermoplastic starch [13] has been reported.

4.4. Solubility in water

As result in Figure 4 clay decreased solubility in water of nanocomposite films. This is mainly due to formation of strong interaction through hydrogen bonds between hydroxyl groups of the biopolymer matrix and nanoclay with high surface area, thus improving the cohesiveness of biopolymer matrix and decreasing the water sensitivity [3]. Decresed in solubility by additional of clay content reported in starch - cmc films [2] and chitosan film [3].

4.5. Transparency

The transparency of film is a desirable property once the consumer wishes to see clearly the aspect of the product which the film will cover. The result shows that transparency of nanocomposite films didn't change significantly. When clay platelets well dispersed through the polymeric matrix since clay platelets are less than the wavelength of visible light and didn't hinder light passage [12, 21]. The similar result reported that nanoclay didn't change transparency of film at low concentration but at high concentration nanoclay wasn't completely dispersed and formed agglomeration in the polymeric matrix and decrease transparency of the films [17].

4.6. SEM micrograph

As shown in Figure 6. nano clay well disturbed into polymeric matrix and preserve the uniformity of the structure of the films. Previous researcher have been shown, if there is affinity between nano particle and polymeric matrix, nano particle can distribute homogeneously [17].

5. CONCLUSIONS

In this study nanoclay was used to improve properties of WPI - PUL film. Nanoclay increased water vapour barrier properties of the biopolymer and reduced water absorption and moisture content. Also results showed that nanoclay led to reduction in water solubility of nanocomposite film. Transparency of the film didn't change significantly due to dispersion of nanoparticles into polymeric matrix.

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REFERENCES

1. ASTM. 1995. Standard test methods for water vapor transition of material, E 95-96. *Annual book of ASTM. Philadelphia, PA: American society for testing and material.*
2. Alamsi H., Ghanbarzadeh B., & Entezami A.A., *Int. J. Biol. Macromol.,* **46** (2010), 1.
3. Casariego A., Souza B.W.S., Cerqueira M.A., Teixeira J.A., Cruz L., Diaz R., *Food Hydrocolloid*, **23** (2009), 1895.
4. Cyras V.P., Manfredi L.B., Ton-That M.T., & Vazquez A., *Carbohydr. Polym.,* **73** (2008), 55.
5. De Wit J.N., *J. Dairy Sci.,* **81** (1998), 597.
6. Ghanbarzadeh B., Almasi H., & Entezami A.A., *Innov. Food Sci. Emerg. Technol.*, (2010), doi:10.1016/j. ifset. 2010. 06.001.
7. Jiang Y., Li Y., Chai Z., & Leng X., *J. Agric. Food Chem.,* **58** (2010), 5100.
8. Kester J.J., & Fennema O., *Food Technol.,* **40** (1986), 47.
9. Kumar P., Sandeep K.P., Alavi S., Truong V.D., & Gorga R.E., *J. Food Eng.,***100** (2010a), 480.
10. Kumar P., Sandeep K.P., Alavi S., Truong V.D., & Gorga R.E., *J. Food Sci.,* **75** (2010b), N46.
11. Lazaridou A., Biliaderis G. & Kontogiorgos V., *Carbohydr. Polym.,* **51** (2003), 151.
12. Ogata N., Jimenez G., Kawai H., & Ogihara T., *J. Polym. Sci., Part B: Polym. Phys.,* **35** (1997), 389.

13.Park H.M., Li X., Jin C.Z., Park C.Y., Cho W.J., & Ha C.S., *Macromol Mater Eng.,* **287** (2002), 553.
14. Park H.M., Liang X., Mohanty A.K., Manjusri M., & Drzal L.T., *Macromolecules*, **37** (2004), 9076.
15.Rao Y.Q., *Polymer*, **48** (2007), 5369.
16.Ray S.S., & Bousmina M., *Mater. Scie.*, **50** (2005), 962.
17.Rhim J.W., Hong S.I., Park H.M., Ng P.K.W., *J. Agric. Food. Chem.,* **54** (2006), 5814.
18.Rhim J.W., *Carbohydr. Polym.,* **86** (2011), 691.
19.Sothornvi R., Hong S.I., An D.J., & Rhim J.W., *LWT-Food Sci., Technol.,* **43** (2010), 279.
20. Sozer N., & Kokini J.L., *Trends Biotechnol*, **27** (2009), 82.
21.Tang S., Zou P., Xiong H., & Tang H., *Carbohydr. Polym.,* **72** (2008), 521.
22.Yano K., Usuki A., & Okai A., J. *Polym. Sci., Part A: Polym. Chem.*, **35** (1997), 2289.