Characterization A Novel Antimicrobial Nano Composite Edible Film Based on Salvia Macrosiphon

Azadeh Sadat Shekarabi and Seyed Mohammad Davachi
Department of Chemical and Polymer Engineering, Faculty of Engineering, Central Tehran Branch, Islamic Azad University, P.O. Box 13185-768, Tehran, Iran
Email: {Shekarabi.chemical, mohdvi}@gmail.com

***Abstract*—This work aimed to develop a nano edible antimicrobial coating based on Salvia macrosiphon seed mucilage (SSM) and nanoclay, incorporated with glycerol to evaluate its physical properties. Upon addition of 2% nanoclay physical and mechanical properties were considerably improved and the composite films showed the lowest water vapor permeability (WVP), as well as highest elongation at break and tensile strength, which makes these films great alternatives for food packaging. While E. coli and S. aureus were significantly inhibited by this antimicrobial films. Results indicated that this novel antimicrobial edible film could have great potential for responsive packaging applications.**

***Index Terms*—Antimicrobial film, salvia macrosiphon, nanoclay, biopolymer, food packaging**

I. Introduction

Food packaging protects food from physical, chemical and biological hazards such as moisture, light, gases, aromas, and microorganism, by keeping its quality and prolongs its shelf life. In recent years, considerable research has been conducted to develop and apply bio-based polymers made from a variety of agricultural commodities and/or wastes of food product industrialization. Such biopolymers include starches, cellulose derivatives, chitosan/chitin, gums, proteins (animal or plant-based) and lipids [1]. Edible coatings have been known to protect food products from deterioration by retarding dehydration, suppressing respiration, improving textural quality, and reducing microbial growth. [2]. Edible coatings are defined as thin layers of wax or other materials which can be applied to the surface of a food or between the food layers [3]. Nanoclay, with a high aspect ratio (100–1500) and extremely high surface-to-volume ratios (700–800 m2g-1), present themselves as an excellent candidate for improvement of the mechanical and barrier properties of polymers [4]. Salvia genus, which is generally called Maryam-Goli in the Persian language, belongs to the Nepetoideae of Mentheae tribe in Lamiaceae family [5]. The genus Salvia (Labiatae) contains more than 700 species, 200 of which exist in Iran and probably in the neighboring countries. These species have been found to possess significant biological activities including antibacterial, antimicrobial, antiviral, antitumor, antioxidant, anti-inflammatory and anti-hydrolytic activity. Some species are used traditionally in foods and cosmetics as well [6]. Salvia macrosiphon is a quite abundant and polymorphic plant in Iran and Afghanistan. Salvia macrosiphon is a local small rounded seed, which quickly produces a transparent mucilaginous gum upon wetting by water. It has been traditionally used in Iran for both pharmaceutical and food applications [7]. Many mucilaginous seeds have been used as natural sources for producing food hydrocolloids, which are used for thickening, gelling, film forming and stabilizing foods and pharmaceuticals. S. macrosiphon seed is considered as a good source of high quantity mucilage with high viscosity. [8]. The purpose of using edible films is to provide a barrier against gases and water vapor and also incorporate functional agents such as antimicrobials and antioxidants to the products, which could lead to improving the mechanical properties. The main novelty of this work is the addition of nanoclay to SSM which can improve its overall properties.

II. Materials and Methods

A. Materials

Salvia macrosiphon seeds were purchased from a local market (Tehran Province, Iran), and their impurities were separated during cleaning. Glycerol (LR grade), which was used as a plasticizer, and the wild sage seeds used in this study were obtained from a local market in Tehran, Iran. The nanoclay (Cloisite 15A) used in this investigation was based on natural montmorillonite clay. Cloisite 15A is a natural montmorillonite modified with a quaternary ammonium salt. This nanoclay is extremely hydrophobic [9] and is obtained from Southern Clay (Gonzales, TX).

B. Nanocomposites Film Preparation

About 30 g salvia macrosiphon seeds were sieved (NO: 20) and washed with ethanol (96% w/v) for 5 min under constant stirring. Then ethanol was evaporated and seeds were dried in an oven at 45 ℃. Aqueous SSM was

Manuscript received May 11, 2018; revised August 20, 2018.

extracted from the whole seeds using distilled water (water to seed weight ratio of 25:1). The swelled seeds were then stirred at 1100 rpm, at 45 °C for 15 min to scrape the mucilage layer off the seed surface. Next, the solutions were filtered. Film solution was prepared by slowly dissolving 30% mucilage, and glycerol (5% [w/w]) was added as plasticizer based on SSM weight under constant stirring (750 rpm) at 45 °C for 15 min [4]. Different concentrations of nanoclay (0.5 to 3 g per 100 g of SSM on a dry basis) were added to the solution. Then the emulsion was placed into an ultrasonic to remove the air bubbles. After the preparation of the emulsions, to prepare films for each sample, about 70mL of each emulsion was poured onto Teflon coated plates (40*40 cm). To control the film thickness, the amount of poured solution was the same (300 mL) in each test resulting in films with 0.08±0.01 mm thickness, measured by a micrometer. The samples were then dried at 35 °C in an oven to cast the films. These nanocomposites films containing SSM and nanoclay from 0.5 to 3% were named SSMXNC, and the X shows the nano percentage in the nanocomposites.

III. Results and Discussion

A. Water Vapor Permeability

Water Vapor Permeability (WVP) is one of the most important factors for biodegradable films. Table I shows the Water Vapor Permeability (WVP) for the salvia macrosiphon films with different nanoclay concentrations. The Neat SSM film (SM0) showed higher permeability values comparing the nanocomposite films during 40 days storage time.

TABLE I. WVP Values for SALVIA Macrosiphon Films

Time (Days)	WVP Values (g/h.m.Pa)					
	SM0	SM0.5	SM1	SM1.5	SM2	SM3
1	1.91	1.72	1.23	0.825	0.74	0.71
6	2.61	1.98	1.64	0.97	0.85	0.81
11	2.89	2.18	1.95	1.08	0.9	0.87
15	3.01	2.46	2.18	1.12	0.934	0.91
20	3.78	2.83	2.24	1.32	1.16	1.07
28	3.91	2.98	2.56	1.54	1.26	1.18
35	4.54	3.28	2.87	1.67	1.59	1.43
40	5.39	3.75	3.01	1.93	1.77	1.65

It can be observed that by addition of nanoclay the WVP is gradually decreased and this trend shows an intense decrease upon addition of higher contents. This behavior can be related to hydrophobic/hydrophilic nature of biopolymers, the presence of voids in their structure and addition of hydrophobic nanoclay [10], [11]. It can be observed after 40 days all the samples show a slight increase in their WVP values which is due to the saturation of the matrix and finding the path of transmission by the water vapor.

B. Film Solubility in Water and Density

Solubility in water is one of the major properties of edible films as most of the required applications need a higher level of water insolubility to increase the product integrity and water resistance, therefore increasing the hydrophobicity is one of the possible solutions [4]. Table 2 shows the solubility of the SSM and its nanocomposites in water and their moisture content. The thickness of the films is between 0.068 and 0.072 mm, which is quite acceptable to compare them.

TABLE II. Film Solubility, Moisture Content and Contact Angle of SSM Nanocomposites

Samples	Thickness (mm)	Moisture content (%)	Solubility in water (%)	Density (gr/cm^3)
SM0	0.068	23.73	29.42	1.46
SM0.5	0.069	22.81	28.21	1.46
SM1	0.071	20.49	25.11	1.46
SM1.5	0.072	18.87	22.48	1.47
SM2	0.073	16.41	19.61	1.47
SM3	0.069	13.26	15.34	1.47

At the same pH, the water solubility was measured for all the samples. The water solubility of the SM0 film is relatively 29.42% and the addition of nanoclay the water resistance of these nanocomposite films is increased which could be due to the hydrophobic nature of Cloisite 15A [9]. As the hydrophobicity of the films increased, the moisture content shows the same trend and is increased upon addition of the nanoclay. The density was slightly increased by the addition of the nanoclay content, however, this increase is so small that can be neglected.

C. Mechanical Properties

Mechanical properties of pure SSM and SSM/clay nanocomposites are listed in Table III. Mechanical properties are critical in packaging materials, therefore, the effect of nanoclay addition to the films has an important role that needs to be considered [4].

TABLE III. MECHANICAL PROPERTIES OF NANOCOMPOSITES

Samples	Elastic Modulus (MPa)	Ultimate Tensile Strength (MPa)	Elongation at break (%)
SM0	327.11±10	16.35±1	5.39±0.2
SM0.5	341±9	18.62±1.6	5.42±0.1
SM1	356±12	21.43±1.3	5.92±0.1
SM1.5	374±11	23.38±1.4	6.43±0.3
SM2	392±12	25.84±2	7.64±0.2
SM3	402±15	24.15±1.3	5.26±0.4

According to the data, ultimate tensile strength, modulus, and elongation at break all increased upon addition of nanoclay until 2%, however with the addition of 3% nanoclay, all the mechanical properties except the modulus decreased. The observed decrease in the mentioned properties is due to the agglomeration of nanoclay. It can be concluded that addition of nanoclay up to a certain percentage enhances the mechanical properties of the SSM nanocomposites most probably due to the strong interactions among nanoclay and SSM which is originated from a reduced free-volume and limited molecular mobility of the polymer chains [4], [12]

D. Antimicrobial Activity

To investigate the antibacterial activity of SSM nanocomposite films in the presence of nanoclay, SM1 as a representative was placed on the seeded agar plates of E. coli and S. aureus on both sides to measure the effectiveness of the films. The test confirms antibacterial activity against both Gram-negative and Gram-positive bacteria on both sides of the film, while shows it has a weak antibacterial activity comparing to the traditional antibacterial agents in lower nanoclay contents.

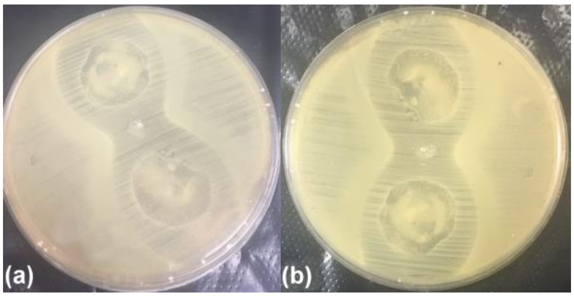

Figure 1. Antibacterial activity against (a) E. coli (b) S. aureus

IV. CONCLUSIONS

It was observed during the present study that the Salvia macrosiphon seed shows a suitable potential to make a good hydrocolloid. The addition of nanoclay up to certain level was shown to improve the mechanical properties including tensile strength, elastic modulus, and elongation at break of the composite films and decrease their permeability. The SSM based nanocomposite films also showed antibacterial activity which could be a useful option while using in packaging applications.

REFERENCES

[1] A. Mitelut, E. Tanse, V. Popa, and M. Popa, "Sustainable Alternative for food packaging" *Chitosan Biopolymer, AgroLife Scientific Journal*, vol. 4, no. 2, 2015.

[2] C. Hana, Y. Zhao, S. W. Leonard, and M. G. Traber, "Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria* × *ananassa*) and raspberries," *Postharvest Biology and Technology*, vol. 33, pp. 67–78, 2004.

[3] V. Bifani, C. Ramírez, M. Ihl, M. Rubilar, A. García, and N. Zaritzky, " Effects of murta (Ugni molinae Turcz) extract on gas and water vapor permeability of carboxymethylcellulose-based edible films," *LWT-Food Sci. Technol*., vol. 40, pp. 1473–1481, 2007.

[4] A. S. Shekarabi, A. R. Oromiehie, A. Vaziri, M. Ardjmand, and A. A. Safekordi, "Investigation of the effect of nanoclay on the properties of quince seed mucilage edible films," *Food Sci. Nutr.*, vol. 2, pp. 821–827, 2014.

[5] M. Karami, M. M. Shamerani, E. Hosseini, A. R. Gohari, M. A. Ebrahimzadeh, and A. Nosrati, "Antinociceptive activity and effect of methanol extracts of three salvia spp. on withdrawal syndrome in mice," *Adv. Pharm. Bull.*, vol. 3, p. 457, 2013.

[6] L. C. Lu, Y. W. Chen, and C. C. Chou, "Antibacterial activity of propolis against Staphylococcus aureus," *Int. J. Food Microbiol*., vol. 102, pp. 213–220, 2005.

[7] S. M. Razavi, A. Bostan, and R. Rahbari, "Computer image analysis and physico-mechanical properties of wild sage seed (Salvia macrosiphon)," *Int. J. Food Prop*., vol. 13, pp. 308–316, 2010.

[8] S. M. Razavi, H. Taheri, and L. A. Quinchia, "Steady shear flow properties of wild sage (Salvia macrosiphon) seed gum as a function of concentration and temperature," *Food Hydrocoll.*, vol. 25, pp. 451–458, 2011.

[9] M. Jafari, S. M. Davachi, J. Mohammadi-Rovshandeh, and P. Pouresmaeel-Selakjani, "Preparation and characterization of bionanocomposites based on benzylated wheat straw and nanoclay," *J. Polym. Environ*., pp. 1–13, 2017.

[10] W. Rhim, S. I. Hong, and C. S. Ha, "Tensile, water vapor barrier and antimicrobial properties of PLA/nanoclay composite films," *LWT-Food Sci. Technol*., vol. 42, pp. 612–617, 2009.

[11] M. B. Vásconez, S. K. Flores, C. A. Campos, J. Alvarado, and L. N. Gerschenson, "Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings," *Food Res. Int.*, vol. 42, pp. 762–769, 2009.

[12] S. Azarmgin, B. Kaffashi, and S. M. Davachi, "Investigating thermal stability and flame retardant properties of synthesized halloysite nanotubes (HNT)/Ethylene Propylene Diene Monomer (EPDM) nanocomposites," *Int. Polym. Process*., vol. 30, pp. 29–37, 2015.

Azadeh Sadat shekarabi: She is an assistant professor of chemical and polymer engineering at Central Tehran Branch Azad University of Iran. She has taught polymer and chemical engineering courses and has number of courses on Food science over the years. Her research and publication interests include biodegradable food packaging, edible films and bio/nanocomposites.

Seyed Mohammad Davachi: He is an assistant professor of chemical and polymer engineering at Central Tehran Branch Azad University of Iran. He got his PhD in polymer engineering in 2016 from University of Tehran and since then joined Central Tehran branch. He has taught both polymer and chemical engineering courses including polymer chemistry and processing, organic

chemistry and heat transfer. He is also active in many research projects and has published more than 10 papers in highly rated journals in 2017. His main interests are biopolymers and biomedical polymers, bio/nano composites, drug delivery systems, polymer synthesis and characterization and surface modification of the polymers.