Enhanced Knowledge in Sciences and Technology Vol. 3 No. 2 (2023) 442-451

EKST

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/ekst
e-ISSN : 2773-6385

Mechanical and Physical Properties of Chitosan Film Incorporated with Plant Oils

Nur Afiqah Nistion[1], Munira Zainal Abidin[1]*

[1] Department of Technology and Natural Resources,
Faculty of Applied Sciences and Technology,
Universiti Tun Hussein Onn Malaysia (Pagoh Campus),
84600 Pagoh, Muar, Johor, MALAYSIA

*Corresponding Author Designation

DOI: https://doi.org/10.30880/ekst.2023.03.02.050
Received 16 January 2023; Accepted 19 February 2023; Available online 30 November 2023

Abstract: Chitosan is notable for its biodegradability, film forming and non-toxicity properties that extracted from shrimp's exoskeletons and other crustaceans. Chitosan film have high water vapor transmission rate (WVTR) and low tensile strength. This research was conducted to prepare edible film with incorporation of plant oils that are olive oil (OO) and lemon essential oil (LO) and analyse the mechanical and physical properties of the films. Variation in chitosan films were produced using casting method on petri dish and dried in drying oven. Film samples were analysed for color, opacity, film solubility, water vapor permeability (WVTR), water vapor permeability (WVP), tensile stress, elongation and Fourier transform infrared spectroscopy (FTIR) analysis. Chitosan film incorporated with olive oil (COO) has greenish color while chitosan film incorporated with lemon essential oil (CLO) was more yellow color than pure chitosan film (CS). Results showed that, film opacity of COO and CLO increase by 53.22% and 162.90% respectively. Film solubility decreases with concentration of OO and LO (8.03% and 18.05%). The WVTR of COO and CLO decreases by 20.99% and 35.85% respectively while WVP decreases by 20.85% and 13.77%. Tensile stress of CLO increases as concentration increases by 109.83%. Elongation of COO decreases (45.59%) while elongation of CLO increases (7.01%) when increased in concentration of oils. FTIR analysis was done to determine bonds and functional groups presence in CS, COO and CLO. Through the analyses, incorporation of plant oils has enhanced the WVTR and tensile stress of chitosan film.

Keywords: Edible Film, Chitosan, Olive Oil, Lemon Essential Oil

1. Introduction

Chitosan, the world's second most abundant biopolymer after cellulose, is notable for its biodegradability, film-forming properties and non-toxicity [1]. In order to reduce waste, several studies and new inventions in food science and technology have been conducted in order to maximize use all parts of raw material from animals and plants while implementing all of the beneficial properties. Edible

*Corresponding author: muniraza@uthm.edu.my

film made of chitosan can efficiently use waste resources such as seafood shells. Chitosan is a protein-rich compound extracted and processed from the exoskeletons of shrimp and other crustaceans. Chitosan has antibacterial and good barrier properties that can help to prolong the shelf life of final food products.

Edible film from chitosan is widely used in the preservation of fresh foods such as meat and meat products, fruits and processed foods [2]. A solid sheet casted to produce edible film that will be wrapped around to cover food product. The edible film and coating's thickness is typically not more than 0.3 mm [3]. If not consumed, edible coatings and films are preferred as food packaging due to their biodegradable and environmentally friendly properties. The edible film is made of food-safe materials and can come into direct contact with the food surface. It can also be combined and interact with other activated compounds, antioxidant and antimicrobial agents to improve the film's functional properties for enhanced food preservation [4].

Plant oils are triglycerides produced from plant sources by extracting oil-producing plant parts. Plant oils are classified as vegetable oils, cooking oils, and essential oils. Natural antimicrobial and antioxidant agents include essential oils (EO) [1]. Among the phenolic chemicals commonly found in essential oils that have the good antibacterial effects to fight with foodborne pathogens are carvacrol, eugenol (2-methoxy-4-(2-propenyl) phenol), and thymol [5].

There has been some study on chitosan combined with plant oils in recent years. The mechanical and physical properties of edible films have been improved by the use of essential oils such as rosemary essential oil (REO) [6], citrus lemon oil [7], lemon essential oil [8], [1], and false flax seed oil [9]. Current research is being conducted to assess the efficacy of incorporating plant oils and their influence on physical and mechanical properties. According to previous study, water vapor transmission rate (WVTR) of chitosan edible film is very high. Chitosan is a biopolymer with a three-dimensional structure that allows it to absorb and maintain storing large amounts of water without completely dissolved [10], [11]. The high WVTR is unsuitable for food packaging because it allows more water content movement between the surrounding environment and the food products. As a result, the purpose of this study is to analyze the effect of lemon essential oil and olive oil on the mechanical and physical properties of chitosan film. In previous study also found when tensile stress is applied to the chitosan film, it fractures or breaks due to its low tensile strength. Chitosan incorporated with lipid should increases tensile stress, as measured by mechanical property analysis.

The goals of this research are to make edible film by combining lemon essential oil (LO) and olive oil (OO) in chitosan solution. This study also looked at the mechanical and physical properties of chitosan films that had been infused with olive oil and lemon essential oil.

2. Materials and Methods

2.1 Materials Specifications

Materials used in this study were chitosan powder (85% degree of deacetylation) was purchased from Medigene Sdn, Bhd. (Selangor, Malaysia), glacial acetic acid (CH_3COOH), deionized water, pure olive oil (OO) was obtained from Lam Soon Edible Oils Sdn. Bhd. (Andalucia, Spain), distilled water, glycerol, Tween 80, dried anhydrous calcium chloride ($CaCl_2$), lemon essential oil (LO) was purchased from Hana Nature (Selangor, Malaysia) and sodium chloride (NaCl).

2.2 Sample preparation

Chitosan films are prepared with some addition and modification from Nazurah et. al. [12]. 2 % w/v of chitosan film was prepared by dissolving chitosan powder in aqueous solution of glacial acetic acid with concentration 1 % v/v being stirred using magnetic stirrer on the hot plate for 20 minutes with 1250 rpm. Glycerol is added to the solution as plasticizer at 25 % w/w of chitosan. After the temperature reached 60 ℃ ($\pm$ 5 ℃) , the stirring is continued for another 20 minutes. The solution removed from hot plate and let cold until temperature drop to 40 ℃ The color of solution formed after chitosan have completely dissolved was yellowish clear solution. This experiment has three types of chitosan film, pure chitosan film as control sample (CS), chitosan incorporated with lemon essential oil (CLO) film

and chitosan incorporated with olive oil (COO) film. COO and CLO were prepared in three sets which are by adding 0.5 %, 1.0 %, and 1.5 % v/v olive oil and lemon essential oil to solutions. The concentration of plant oils incorporated into the chitosan solution were varied in this study. The COO and CLO films were then treated with 0.2 % w/v Tween 80 as an emulsifier to prevent or reduce separation of chitosan and plant oils. For all samples, the polymer solution is homogenized using a homogenizer at a fixed 10 000 rpm for 10 minutes [13].

CLO and COO solution was degassed in ultrasonic bath (Elmasonic S30H, United State) for 15 minutes at 35 ℃ to remove air bubbles present due to vigorous homogenizing speed. The solutions were cold before poured into the middle of 9 cm diameter Petri dishes with volume 20 ml. The film-forming solutions were dried in drying oven (Memmert UF160, Germany) at 50℃ ($\pm$ 5 ℃) for 18 hours. After drying, the dried films were peeled and stored in a desiccator at 25 ℃ for another 48 hours [7]. By standardizing the volume of chitosan blend solution poured (20 ml) on Petri dishes and the size of Petri dishes used in diameter and height dimensions, the thickness of edible films remained uniform (9 cm diameter). The thickness of film samples is measured with a micrometer with a precision of 0.01 mm at five different random positions on each type of sample. The average thickness of the film will be used to calculate opacity, tensile stress, elongation, and water vapor permeability (WVP) [13].

2.3 Physical properties of chitosan edible film

2.3.1 Film color

A colorimeter (Miniscan EZ 4000 Hunter Lab, Virginia, United State) is used to determine the film color of each sample in aspects L* for brightness, a* for greenness (negative value) or redness (positive value), and b* for blueness (negative value) or yellowness (positive value). Color was measured with three readings on the film to obtain average color values and recorded [14].

2.3.2 Film opacity

The opacity of edible films was determined using a UV-VIS spectrophotometer (PG Instruments Model T60, UV-VIS Spectrophotometer, United Kingdom) by placing rectangular strips inside the spectrophotometer's cuvette. In the batch of samples, an empty clear cuvette was used as a reference. UV-Visible spectrophotometer absorption spectrum of samples at 600 nm. The readings were taken in triplicate to obtain the average value of the opacity of the films. The opacity can be calculated by using Eq. 1 [15].

$$Opacity\ (nm^{-1}) = \frac{absorbance\ 600\ nm}{sample\ thickness\ (mm)} \qquad Eq.\ 1$$

2.3.3 Film solubility

The water solubility of the control sample, CLO, and COO was determined using the gravimetric method at 25 $\pm$ 1 °C. The film samples were cut into a rectangular shape measuring 2×2 cm. To obtain the initial dry mass, the samples were dried in a drying oven at 70 °C for 24 hours (M1). After drying for 24 hours, the samples were placed in 30 mL of distilled water and stored at room temperature (25 $\pm$ 2 °C) for 24 hours. After 24 hours in distilled water, samples were removed and dried at 70 °C for another 24 hours. The dried samples were weighed (M2), and the percentage of water solubility was calculated using Eq. 2. The test is repeated three times to obtain the average value as the results [16].

$$\text{Water solubility } \% = \frac{initial\ weight\ (M1) - final\ weight(M2)}{initial\ weight\ (M1)} \times 100 \qquad Eq.\ 2$$

2.3.4 Water vapor permeability (WVP) and water vapor transmission rate (WVTR)

The WVTR and WVP analyses were performed in accordance with the universal standard method ASTM E96. The gravimetric cup method was used to determine the WVTR of the films. The film samples were sealed on top of a crucible containing 1 g of dried anhydrous calcium chloride with an internal diameter of 0.02 m and an exposed area of 3.14×10^{-4} m^2. At 25 °C, the weight of the film sealed on the jar was recorded and placed in a desiccator containing saturated sodium chloride, NaCl aqueous solution. Because of the presence of anhydrous calcium chloride, the relative humidity inside

the jar is nearly 0 %, while the external relative humidity is 75 % due to the NaCl solution. Moisture enters the jar only through the film in this sealing condition. For 5 days, the difference in jar weight is measured and recorded every 24 hours. Eq. 3 and 4 can be used to calculate the WVTR and WVP of film samples [17].

$$WVTR = \frac{w}{A \times t} \quad Eq.\ 3$$

$$WVP = \frac{WVTR \times d}{p \times (R_1 - R_2)} \quad Eq.\ 4$$

Where w is the mass gained (g), t is the duration (s), A is the film area exposed for water vapour transmission (m^2), d is the thickness of the film (m), p is the saturation vapour pressure of water (3171.8 Pa at 25 °C), R_1 is the relative humidity outside the jar (75 %), and R_2 is the relative humidity inside the jar (0 %).

2.4 Mechanical properties of chitosan edible film

2.4.1 Tensile stress

A texture analyzer is used to determine the tensile stress of chitosan films (Stable MicroSystem, model TA.XT.Plus, United Kingdom). The measurement of film strip that were analyzed were 20 × 70 mm. The initial grip separation and crosshead speed settings are set to 50 mm and 0.8 mm s^{-1}, respectively [8]. Tensile stress values were calculated using Eq. 5.

$$Tensile\ stress = \frac{Force\ (N)}{Cross\ sectional\ area\ (m^2)} \quad Eq.\ 5$$

2.4.2 Elongation

Elongation at break was calculated by dividing elongation at the point of rupture by the length of the original sample, multiplying by 100, and expressing the result as a percentage of the original length [18]. Elongation of film similar to elongation at break except force applied extend the film without breaking. Elongation can be calculated using the same formulation. Elongation percentage was calculated using Eq. 6.

$$\text{Elongation } (\%) = \frac{length\ final - length\ initial}{length\ initial} \times 100\% \quad Eq.\ 6$$

2.5 Fourier transform infrared spectroscopy (FTIR) analysis

The presence of chitosan-olive oil complex and chitosan-lemon essential oil complex in the sample was determined using FTIR analysis. This analysis was performed with a fourier-transform infrared spectroscopy machine (Agilent Technologies, Cary 630 FTIR Spectrometer, United State). The film samples were cut into square (1 cm ×1 cm) shapes and observed at wavelengths ranging from 4000 to 800 cm^{-1} [1]. FTIR Spectrum Software was used to scan the samples and evaluate the results.

2.6 Statistical analysis

SPSS software's one-way analysis of variance (ANOVA) is used to evaluate the results in mean standard deviation of data with some modification. There are three sample variables in this study: chitosan control sample, CLO film, and COO film, with three replications [1, 7].

3. Results and Discussion

3.1 Film color

Color is important in food packaging as influence consumers acceptance of the films. As shown in Table 1, pure chitosan film (CS) has high brightness, L* value 75.21, some hint of green color and yellow color. The mixture of chitosan with acetic acid and glycerol produced clear yellow solution.

Based on L* values, the brightness of COO and CLO film decreases as the oils' concentration increases. COO films have high in green color compared to other samples and the negative a* value indicating green color gradually increased with concentration. Green color of COO films came from color of OO used that contained chlorophyll and polyphenol content. CLO films have decreased in a* value but increased b* value when more LO incorporated. CLO have high in yellow color intensity compared to CS and COO films. Yellow color of CLO film obtained from color of LO used in this study. One way ANOVA using SPSS software with level of significance 95 % shows there was significant difference between all samples with $p<0.05$.

Table 1: Color measurement of pure chitosan film (CS), chitosan film incorporated with olive oil (COO) and lemon essential oil (CLO)

Samples	Color measurement		
	L*	a*	b*
CS	75.21±0.42	-1.89±0.28	11.52±0.46
0.5 COO	34.58±1.91	-2.69±0.06	4.44±0.50
1.0 COO	33.54±0.36	-2.76±0.12	3.57±0.21
1.5 COO	27.73±1.35	-2.98±0.13	3.10±0.09
0.5 CLO	82.94±1.38	-2.26±0.18	17.34±0.49
1.0 CLO	82.22±1.11	-1.85±0.17	18.21±0.57
1.5 CLO	75.38±1.21	-1.40±0.31	19.22±1.39

*Data are expressed as the Mean ±Standard Deviation (SD)

3.2 Film opacity

Opacity attribute affect the rate of photooxidation process to occur in food products. Higher opacity can help to limit amount of light passes through film that act as packaging barrier. Based on Table 2, CS have the lowest opacity value compared to COO and CLO films. The opacity of COO and CLO film increases when concentration of oils increases. 1.5 COO film have the highest opacity value can be efficiently used to block light from food products. The transparency of film impacted by oil droplets spread in chitosan matrix causes light blocked from passing through the formed film [13]. The opacity level also affected by types of lipids added and initial composition of film forming dispersion gave impact on structure that emerges during drying process. There was significant difference between sample ($p<0.05$) based on one way ANOVA.

Table 2: Physical properties of pure chitosan film (CS), chitosan film incorporated with olive oil (COO) and lemon essential oil (CLO)

Samples	Thickness (mm)	Opacity (nm^{-1})	Solubility (%)	WVTR (g/m^2 s)	WVP ($\times10^{-12}$ g/m s Pa)
CS	0.08±0.01	0.804±0.01	32.50±2.11	0.0129±0.00025	4.3494±0.08461
0.5 COO	0.11±0.01	3.570±0.01	19.31±1.74	0.0081±0.00010	3.7351±0.04798
1.0 COO	0.10±0.02	4.700±0.01	18.62±0.43	0.0076±0.00009	3.1753±0.03712
1.5 COO	0.11±0.03	5.470±0.01	17.76±0.29	0.0064±0.00017	2.9562±0.07896
0.5 CLO	0.09±0.02	1.922±0.07	25.98±1.56	0.0106±0.00015	3.9977±0.05778
1.0 CLO	0.10±0.02	1.920±0.00	24.98±1.15	0.0085±0.00022	3.5661±0.09165
1.5 CLO	0.12±0.05	5.053±0.09	21.29±1.41	0.0068±0.00019	3.4471±0.09603

*Data are expressed as the Mean ±Standard Deviation (SD)

3.3 Film solubility

Plant oils have hydrophobic property that will repel water molecules from forming bonds with its compound. Incorporation of plant oils can reduce film solubility in water. In this study, CS film have the highest solubility which was 32.50 % indicating it highly soluble in water. When concentration of COO and CLO increased, the solubility of films were decreased as shown in Table 2. Interaction

between LO and chitosan created cross-linking effects that cause more rigid structure formed [8]. Solubility of COO film improved by compact and continues structure of COO film that can be observed from SEM analysis. Interaction between carboxylate groups of OO and amino groups of chitosan were too strong for water molecule to form bonds with amino groups of chitosan chains [16]. There was significant difference between each sample ($p<0.05$) when analyzed using ANOVA in SPSS software.

3.4 Water vapor transmission rate (WVTR) and water vapor permeability (WVP)

Table 2 shows incorporation of OO and LO into chitosan film which have effectively reduced the WVTR and WVP by limiting water molecules penetrate into films. At 1.5% concentration of OO and LO incorporated, 1.5 COO has lower WVTR which is 0.0064 g/m^2 s compared to 1.5 CLO (0.068 g/m^2 s). This showed OO was better in lowering the WVTR and WVP. LO can lower the WVTR and WVP because the oil droplets dispersion in chitosan film matrix may create a tortuosity channel that slow the movement of water vapor molecules through the film [19]. Tortuosity is characterization of porous material indicating ratio of actual flow pathlength travel to the straight distance between both end of length. There was significant difference in each variation of chitosan film ($p<0.05$) when analyzed using ANOVA.

3.5 Tensile stress of films

Tensile stress was the force per unit area that related to stretching and elongation of material without breaking film samples. Tensile strength is the maximum amount of tensile stress can be applied on material before it breaking. Thus, increasing in tensile stress shows increase in tensile strength value. CS have the highest tensile stress which is 32222.23 Nm^{-2} while 0.5 CLO have the lowest tensile stress 7642.90 Nm^{-2} as shown in Table 3. High tensile stress refers to more force required for film's elongation. 0.5 CLO have potential of low durability because it can break easily compared to other films when little forced was applied. Increasing in OO concentration produced stronger COO film with strong intramolecular force as more force required to stretch COO film. OO has high in monounsaturated fatty acids (oleic acid) that can increase flexibility and extensibility. LO increase the tensile stress of chitosan film by creating cross-linker interaction with other components to reduce polymer molecular mobility by fixing the polymer's structure [20].

Table 3: Mechanical properties of pure chitosan film (CS), chitosan film incorporated with olive oil (COO) and lemon essential oil (CLO)

Samples	Tensile stress (Nm^{-2})	Elongation (%)
CS	32222.23±5341.494	35.25±0.25
0.5 COO	13788.27±2211.916	35.36±0.22
1.0 COO	21069.83±3518.235	35.36±0.08
1.5 COO	20137.87±3382.539	19.24±8.28
0.5 CLO	7642.90±199.799	38.22±4.84
1.0 CLO	14156.93±2741.178	41.22±0.43
1.5 CLO	16037.40±2834.944	40.90±0.12

*Data are expressed as the Mean ±Standard Deviation (SD)

3.6 Elongation

Elongation of films measured with length of film before and after force applied at the both end of film strips to pull it apart. The percentage of elongation were calculated to determine the flexibility of films. According to Table 3, CLO film have high elongation percentage indicate it have high flexibility compared to CS and COO film. The elongation percentage of CLO increase with increasing in LO concentration. Sample 1.5 COO film have low flexibility compared to other COO film because two of the tested samples broken when reached maximum force applied. Further increase in OO concentration create too strong intermolecular and intramolecular force causes 1.5 COO film to break. This result similar with quinoa protein-chitosan film incorporated with sunflower oil increased the extensibility at

first, but decreased extensibility when higher concentration of sunflower oil was added [21]. Thus, elongation of chitosan film depends on type of oils used. According to ANOVA analysis with 95% level of significance, there were significant difference ($p<0.05$) between CS, COO and CLO film.

3.7 Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analysis was carried out to determine the films features and functional groups presence in the compound of film. The functional groups' presence in compounds were determined by position of peaks at absorption region. Absorbance of FTIR refers to amount of light absorbed when it passes through sample.

Figure 1: FTIR spectrum of pure chitosan film (CS)

In pure chitosan film (CS) as shown in Figure 1, there were broad peak of O-H stretching at 3210.902 cm^{-1}, H-C-H stretching at 2925.901 cm^{-1} and 2878.642 cm^{-1}, C=O stretching at 1635.725 cm^{-1} and N-H bending at 1541.782 cm^{-1}. There also C-H bending at 1405.323 cm^{-1}, C-N stretching at 1151.141 cm^{-1}, and strong sharp peak at 1065.440 cm^{-1} and 1020.889 cm^{-1} of C-O stretching. The presence of acetic acid can be detected with O-H stretching of carboxylic acid within range 3000-2500 cm^{-1} with strong and broad peak. Chitosan compound were detected when C=O stretching and N-H bending of amines presence in the compound. The C-O stretching was corresponding to presence of alcohol (glycerol) in the film compound.

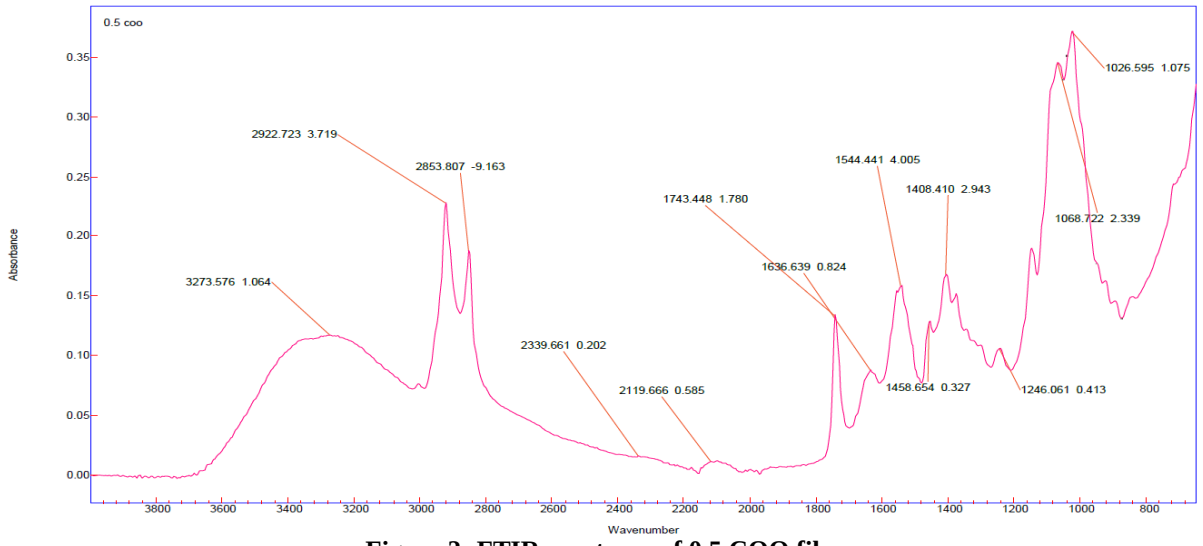

Figure 2: FTIR spectrum of 0.5 COO film

Figure 2 shows FTIR spectrum of chitosan film incorporated with 0.5 wt% olive oil (0.5 COO). In COO films, the peaks detected at wavenumber range similar to CS film. All COO film has O-H stretching at in range 3254-3273 cm^{-1} of carboxylic acid corresponds to acetic acid. 0.5 COO film has sharp peak of H-C-H stretching at 2922.723 cm^{-1} and 2853.807 cm^{-1} because of long chain of unsaturated fatty acids incorporated into chitosan film [16]. This sharp peak of H-C-H stretching decrease when concentration of olive oil increases. There was new peak in range 1743-1744 cm^{-1} of C=O shows presence of Tween 80 and fatty acids.

Figure 3: FTIR spectrum of 0.5 CLO film

Figure 3 shows FTIR spectrum of chitosan film incorporated with 0.5 wt% lemon essential oil (0.5 CLO). In all CLO films, there was H-C-H stretching in range 2925-2875 cm^{-1} shows presence of lipid carbohydrate in the compounds [1]. The N-H bending of all CLO films located at 1543 cm^{-1} showing presence of chitosan compound. The peak of C=C at range 1637-1635 cm^{-1} show presence of LO and Tween 80 in the compound.

Table 4: Summary of ANOVA for descriptive test

Analyses	Sum of Square	Degree of freedom (df)	Mean Square	F-value	P-value	
Color L*	11597.451	6	1932.908	1303.908	0.000	Significant
Color a*	6.027	6	1.004	26.010	0.000	Significant
Color b*	962.042	6	160.340	371.203	0.000	Significant
Opacity	59.483	6	9.914	5798.831	0.000	Significant
Solubility	498.821	6	83.137	43.012	0.000	Significant
WVTR	0.000	6	0.000	512.702	0.000	Significant
WVP	4.072	6	0.679	124.907	0.000	Significant
Tensile stress	1079303975	6	179883995	17.323	0.000	Significant
Elongation	997.214	6	166.02	12.599	0.000	Significant

4. Conclusion

Chitosan film incorporated with olive oil (COO) and lemon essential oil (CLO) were successfully produced. Seven types of samples were produced, pure chitosan sample (CS), COO and CLO film with three different concentrations (0.5, 1.0 and 1.5%). It can be concluded that by increasing in concentration of plant oils have increase film opacity to limit light pass-through sample, reduced film solubility in water, reduced water vapor transmission rate (WVTR) and water vapor permeability (WVP). Important step in preparing chitosan film involved dissolve chitosan powder, homogenizing

with plant oils and degassed step. Homogenizing important to prevent oil separation on the film surface. The air bubbles in chitosan mixture solution were removed after homogenizing before drying. The most efficient concentration was recognized and can be further study. 1.5 COO and 1.5 CLO film were the most efficient film to be used in enhancing chitosan film opacity, solubility, WVTR and WVP. As for tensile stress and elongation properties, 1.0 COO and 1.0 CLO were more efficient than other samples since the films can withstand high tensile stress and have high elongation percentage without break. Therefore, the objectives of this study were achieved by chitosan films produced and analyses conducted. For future study suggestion, scanning electron microscopy (SEM) analysis can be done to observed microstructure of film. Antibacterial activity and oxygen permeability analysis also can be conducted to study the effectiveness of chitosan film incorporated with plant oils in preventing microbial growth and oxidation process. Further study on chitosan film as packaging of sugar and coffee powder where does not require to remove the packaging also can be done.

Acknowledgement

The authors would also like to thank the Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia for providing experimental facilities.

References

[1] Tügen, A., Ocak, B., & Özdestan-Ocak, Ö. (2020). Development of gelatin/chitosan film incorporated with lemon essential oil with antioxidant properties. *Journal of Food Measurement and Characterization*, *14*(6), 3010-3019

[2] Umaraw, P., & Verma, A. K. (2017). Comprehensive review on application of edible film on meat and meat products: An eco-friendly approach. *Critical reviews in food science and nutrition*, *57*(6), 1270-1279.

[3] Jeevahan, J. J., Chandrasekaran, M., Venkatesan, S. P., Sriram, V., Joseph, G. B., Mageshwaran, G., & Durairaj, R. B. (2020). Scaling up difficulties and commercial aspects of edible films for food packaging: A review. *Trends in Food Science & Technology*, *100*, 210-222.

[4] Jahantigh, F. (2020). Edible film based on Vicia faba shell gum, nano silver and Helichrysum arenarium essence. *Nutrition & Food Science*, *50*(2), 280-287.

[5] Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International journal of food microbiology*, *94*(3), 223-253.

[6] Abdollahi, M., Rezaei, M., & Farzi, G. (2012). Improvement of active chitosan film properties with rosemary essential oil for food packaging. *International journal of food science & technology*, *47*(4), 847-853.

[7] de Oliveira Filho, J. G., de Deus, I. P. B., Valadares, A. C. F., Fernandes, C. C., Estevam, E. B. B., & Egea, M. B. (2020). Chitosan film with citrus limonia essential oil: physical and morphological properties and antibacterial activity. *Colloids and Interfaces*, *4*(2), 18.

[8] Peng, Y., Yin, L., & Li, Y. (2013). Combined effects of lemon essential oil and surfactants on physical and structural properties of chitosan films. *International Journal of Food Science & Technology*, *48*(1), 44-50.

[9] Gursoy, M., Sargin, I., Mujtaba, M., Akyuz, B., Ilk, S., Akyuz, L., Kaya, M., Cakmak, Y. S., Salaberria, A. M., Labidi, J., & Erdem, N. (2018). False flax (Camelina sativa) seed oil as suitable ingredient for the enhancement of physicochemical and biological properties of chitosan films. *International journal of biological macromolecules*, *114*, 1224-1232.

[10] Sánchez-Machado, D. I., López-Cervantes, J., Correa-Murrieta, M. A., Sánchez-Duarte, R. G., Cruz-Flores, P., & de la Mora-López, G. S. (2019). Chitosan. In *Nonvitamin and nonmineral nutritional supplements* (pp. 485-493). Academic Press.

[11] Xie, H., Jia, Z., Huang, J., & Zhang, C. (2011). Study on the preparation of superabsorbent composite of chitosan-g-poly (acrylic acid)/kaolin by in-situ polymerization. *International Journal of Chemistry*, *3*(3), 69.

[12] Nazurah, R. N. F., Noranizan, M. A., Nor-Khaizura, M. A. R., & Hanani, Z. N. (2022). The potential of chitosan-based film with curry leaf essential oil as natural insect-repellent food packaging. *Food Packaging and Shelf Life*, *34*, 100993.

[13] Pereda, M., Amica, G., & Marcovich, N. E. (2012). Development and characterization of edible chitosan/olive oil emulsion films. *Carbohydrate polymers*, *87*(2), 1318-1325.

[14] Thakhiew, W., Devahastin, S., & Soponronnarit, S. (2013). Physical and mechanical properties of chitosan films as affected by drying methods and addition of antimicrobial agent. *Journal of food engineering*, *119*(1), 140-149.

[15] Souza, V. G. L., Fernando, A. L., Pires, J. R. A., Rodrigues, P. F., Lopes, A. A., & Fernandes, F. M. B. (2017). Physical properties of chitosan films incorporated with natural antioxidants. *Industrial Crops and Products*, *107*, 565-572.

[16] Akyuz, L., Kaya, M., Ilk, S., Cakmak, Y. S., Salaberria, A. M., Labidi, J., ... & Sargin, I. (2017). Effect of Different Animal and Plant Oil Additives on Physicochemical, Mechanical, Antimicrobial and Antioxidant Properties of Chitosan Films. *Smart Bio*, 18.

[17] Priyadarshi, R., Kumar, B., Deeba, F., Kulshreshtha, A., & Negi, Y. S. (2018). Chitosan films incorporated with Apricot (Prunus armeniaca) kernel essential oil as active food packaging material. *Food Hydrocolloids*, *85*, 158-166.

[18] Binsi, P. K., Ravishankar, C. N., & Srinivasa Gopal, T. K. (2013). Development and characterization of an edible composite film based on chitosan and virgin coconut oil with improved moisture sorption properties. *Journal of Food Science*, *78*(4), E526-E534.

[19] Pérez-Gago, M. B., & Krochta, J. M. (2001). Lipid particle size effect on water vapor permeability and mechanical properties of whey protein/beeswax emulsion films. *Journal of agricultural and food chemistry*, *49*(2), 996-1002.

[20] Ojagh, S. M., Rezaei, M., Razavi, S. H., & 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*(1), 161-166.

[21] Valenzuela, C., Abugoch, L., & Tapia, C. (2013). Quinoa protein–chitosan–sunflower oil edible film: Mechanical, barrier and structural properties. *LWT-Food Science and Technology*, *50*(2), 531-537.