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Effect of different animal fat and plant oil additives on physicochemical, mechanical, antimicrobial and antioxidant properties of chitosan films

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ABSTRACT

Practical application of chitosan-essential oil blend films is limited due to the uneconomical extraction procedure of essential oils from plants. This study aimed to produce chitosan films blended with low cost and commercially available oils and fats consumed in daily human diet (olive, corn and sunflower oils, butter and animal fats). The study also focused on how physicochemical, biological and mechanical properties of chitosan blend films were influenced by the incorporation of oils and fats with varying unsaturation degrees. Possible interactions of chitosan film matrix with incorporated oils or fats were investigated. Chitosan-olive oil film showed better surface morphology and higher thermal stability than the films with other unsaturated oils. Tensile strength, Young's modulus and elongation at break were improved by 57.2%, 25.1% and 31.7% for chitosan-olive oil film, respectively. Chitosan-olive oil blend film had the highest antibacterial activity (almost equal to that of commercial antibiotic gentamicin). Edible films obtained from by incorporation of natural oils and fats into chitosan can help produce an environmentally friendly packaging material that is low cost and easily manufactured.

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1. Introduction

With the increase of industrial food consumption in recent years, petroleum-derived polymers have been widely used as a packaging material for extension of shelf life of the food product and prevention of contamination [1]. Unfortunately, food packaging from synthetic petroleum-based polymers (polyolefins, polyesters, polyamides etc.) release monomers that are detrimental to health. Petroleum-based packaging wastes cause environmental pollution because of their stability against decomposition in nature [2, 3]. To overcome this problem, many researchers are working on production of natural and bio-based edible films to replace synthetic petroleum-based polymers [4]. Also, edible films can contribute to the nutritional value of the content and can extend its shelf life by reducing gas and vapor permeability [5, 6].

Edible films are the biological materials that are obtained from natural sources such as polysaccharides, proteins, lipids and plant extracts or their combination [4]. Various types of protein such as gluten, gelatin, soy protein, milk protein, whey protein and wheat protein have been used for the production of edible films as a matrix [4]. However, in the production of edible films polysaccharides (cellulose, starch, gelatin and chitosan) are the most commonly used film preparation material [7]. But, aside from their low gas barrier properties, hydrophilic nature of polysaccharides renders them unsuitable for production of edible films [8]. Nonetheless, the tendency of polysaccharide-based edible films to absorb water can be lowered by addition of several hydrophobic materials such as beeswax and fatty acids.

Thanks to its antioxidant, antimicrobial and biocompatibility properties, chitosan is one of the most commonly used polysaccharide for production of edible films [9]. Chitosan is a natural biopolymer derived from deacetylation of chitin, the second most abundant biopolymer after the cellulose [10]. Deacetylation degree and average molecular weight of chitosan are the parameters affecting its antimicrobial and film-forming properties [11]. On the other hand, operational conditions such as pH and solvent type can affect mechanical and physicochemical characteristics of chitosan films. Chitosan films protect the food from fungal degradation as they can form an excellent oxygen barrier. Chitosan films have long life and very good mechanical properties. However, when prepared alone, it is not water resistant due to the numerous H bonds in its structure. This makes it difficult to use chitosan as a moisture barrier. This is a drawback of chitosan films produced to be used as a packaging material, despite its wider applications in pharmaceutical

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products and membrane separation systems. To alleviate this problem, hydrophobic materials including essential oils are incorporated into chitosan films to make them resistant to water and to enhance their protective property. In many studies, composite films were formed by using essential oils extracted from plants such as cinnamon, clove, thyme, fennel, cypress, lavender, pine, rosemary, grape seed and green tea [12–15]. It has been reported that properties of chitosan films can vary according to film morphology, molecular weight and deacetylation degree of chitosan and amount and type of oil added to the film [16]. Previous studies demonstrated that antimicrobial and antioxidant activities and mechanical properties of chitosan films can be greatly improved with addition of natural essential oils [17]. But, extracting essential oils from plants is a difficult and costly process. Effects of olive oil, corn oil and sunflower oil on physicochemical properties of chitosan films were examined in earlier studies [18–20]. However, effect of saturated and unsaturated oils on chitosan films has not been comparatively explained before.

The aim of the present study is to prepare low cost chitosan films blended with saturated animal fats and vegetable oils with varying unsaturation degree. Also the effects of film composition on its mechanical, physicochemical and antimicrobial properties were studied. For this purpose, olive oil, corn oil, sunflower oil, butter and animal fat were used as film additives.

2. Materials and method

2.1. Materials

Chitosan (medium molecular weight, Cas No: 448,877) was purchased from SIGMA-Aldrich, USA. Acetic acid, glycerol and Tween 40 were supplied from MERCK, Germany. Olive oil, corn oil, sunflower oil, butter and animal oil were purchased from a local market.

2.2. Preparation of oil or fat-chitosan blend films

Six different types of the film samples were produced as follows: Chitosan was (600 mg) was dissolved in 60 mL of acetic acid solution (%1 v:v) under constant stirring at 100 rpm at 25 °C for 24 h. After filtration of each solution, 100 μL of glycerol (as a plasticizer) and 10 μL of Tween 40 (as an emulsifier agent) were added into each of chitosan solution. Chitosan solution without oil or fat content was used as a control. For the blend films, 0.15 mL of olive oil, corn oil, sunflower oil, melted butter and melted animal fat were put into respective chitosan solution. The mixtures were homogenized using a homogenizer (Heidolph, Silent Crusher M) at 25.000 rpm for 5 min. Following the homogenization process, all the samples were poured into the plastic Petri dishes (with 15 cm diameter) and dried at 25 °C for 48 h. Images of the films are presented in Fig. 1.

2.3. Characterization of oil or fat-chitosan blend films

2.3.1. Fourier transform infrared spectroscopy

Oil or fat-chitosan blend films were analyzed using by a Perkin-Elmer ATR FT-IR spectrometer in a spectral range of 4000–650 cm^{-1}. Infrared spectra of pure chitosan and free oil/fat samples were recorded to make comparison of the band changes after the preparation of the chitosan blend films.

2.3.2. Thermogravimetric analysis

Thermogravimetric analysis was carried out using a thermogravimetric analyzer (TGA/SDTA 851 Mettler Toledo instrument). The specimens were approximately taken as 5 mg and heated to 650 °C at a heating rate of 10 °C min^{-1} under nitrogen atmosphere (20 mL min^{-1}).

2.3.3. Differential scanning calorimetry

In order to determine thermal properties of the film samples, DSC analysis was performed on a Mettler Toledo DSC822e (Schwerzenbach, Switzerland) under nitrogen atmosphere. Film samples (50 mg) were sealed in hermetic aluminum pans and heated with at a heating rate of 5 °C min^{-1} at a temperature range of −50 to 420 °C.

2.3.4. Scanning electron microscopy

For examination of surface morphologies of the films, scanning electron micrographs were recorded on SEM, Zeiss, Eco 40 (Germany). The samples were coated with gold/palladium as a thin layer before the analysis using Sputter Coater (Cressingto Auto 1808). Surface smoothness and porosity were analyzed.

2.3.5. Mechanical properties

Tensile strength, elongation at break and Young's modulus test were conducted for each film sample on Material Testing Systems (MTS Insight 10) equipment with a load cell of 250 N. Cross-head speeds were

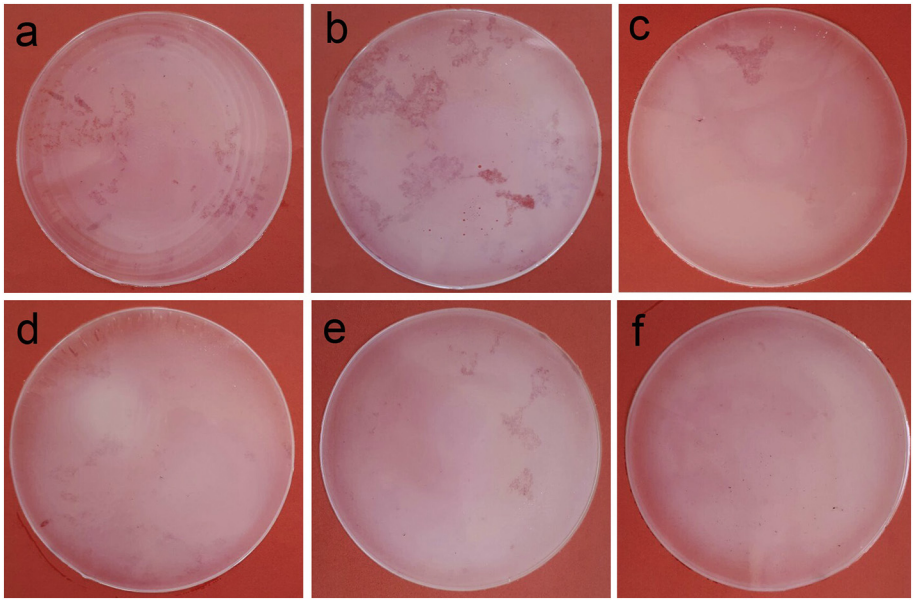


Fig. 1. Appearances of the a) chitosan-control film b) chitosan-olive oil blend film, c) chitosan-corn oil blend film, d) chitosan-sunflower oil blend film, e) chitosan- butter blend film and f) chitosan- animal fat blend film.

chosen as 5 mm min^{-1}. MTS Test Works 4 software was applied for calculating the mechanical properties.

2.3.6. Light transmittance of chitosan-oil/fat blend films

Transparency of chitosan-control and chitosan-oil/fat blend films was examined using a UV spectrophotometer (Shimadzu UV-3600 UV–VIS–NIR) in the light range of 400–700 nm.

2.3.7. Surface hydrophobicity

Surface hydrophobicity of the control (oil or fat free chitosan film) and chitosan-oil/fat blend films was evaluated via contact angle measurements using Data Physics video-based contact angle measurement system OCA20. The sessile drop method, which is the most frequently used method for estimation of wetting properties of edible films, was used to evaluate the contact angle. A drop of distilled water was placed on the film surface and its volume was measured using software controlled dosing volume weight-drop. The shape of water drop was examined and the surface energy was determined. Eight measurements were carried out for each film. An average of the eight measurements was used for determination of wetting behavior of the film samples.

2.4. Water solubility of chitosan-oil/fat blend films

The water solubility (WS) of chitosan-oil/fat blend films was determined gravimetrically at 25 ± 1 °C. The control and chitosan-oil/fat blend films were cut into the rectangular shape (1 × 2 cm). Accurately weighed film samples were placed into 30 mL of distilled water and carefully stirred at different time intervals. After the immersion of 24 h, the samples were recovered and dried at 50 °C for 24 h. Dried samples were weighed and %WS was calculated according to Eq. 1. The tests were repeated for three times and the results were given as average of the three tests.

$$WS\% = (initial\ weight - final\ weight / initial\ weight) x100 \quad (1)$$

2.5. Soil degradation of chitosan-oil/fat blend films

The soil degradation of the film samples was determined according to percentage weight loss (*WL %*) within 15 days. Chitosan-oil/fat blend film samples (in rectangular shape of 2 × 3 cm) were weighed and dried in an oven for 48 h to a constant weight. Then, the dried film samples were weighed again. Recorded weights were taken as the initial weight (M_0). The film samples were buried at the 5 cm under the surface of the soil (pH 6.0–7.0; organic matter content (OMC): 15%; total dissolved solids: 1–2 mS cm^{-1}) in a series of the plastic pots. The samples were incubated at 25 ± 1 °C for 15 days. During the incubation, 10 mL of water was added to the plastic boxes to keep the soil moisture at 40% each day. The samples were recovered from the soil at the end of 15 day-incubation period. The samples were dried in oven at 50 °C and weighed (M_f). The soil degradation of the samples was calculated using the Eq. 2. The tests were repeated thrice and the results were given as average ± SD.

$$WL\% = (M_0 - M_f / M_0) x100 \quad (2)$$

2.6. Biological properties of chitosan-oil/fat blend films

2.6.1. Antioxidant activity

Antioxidant activities of the control and chitosan-oil/fat blend films were determined using Ferric Reducing Ability of Plasma (FRAP) method applied by Aktumsek et al. [21]. Reactive solutions of 300 mM acetate buffer, 10 mM TPTZ and 20 mM ferric (III) chloride solutions were prepared (10:1:1; v:v:v). The film samples (10 mg) were cut into small pieces and placed in test tubes. Two mL of reactive solution was added onto the samples and incubated for 30 min at 30 °C. The amount of Fe^{2+} was determined spectrophotometrically at 593 nm. Troloxane was used as a standard.

2.6.2. Antimicrobial activity

2.6.2.1. Microorganisms used in the study. The food-borne and human pathogen microorganisms (*Escherichia coli* (*E. coli*) ATCC 25922, *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Proteus microbilis* (*P. microbilis*) ATCC 14153, *Proteus vulgaris* (*P. vulgaris*) ATCC 13315, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853, *Enterobacter aerogenes* (*E. aerogenes*) ATCC 13048, *Bacillus thuringiensis* (*B. thuringiensis*), *Salmonella enterica serotype typhmurium* (*S. typhmurium*) SL 1344 and *Streptococcus mutans* (*S. mutans*) ATCC 25175) were used to evaluate the antibacterial properties of chitosan-oil/fat blend films. Bacteria were maintained on Luria Bertoni (LB) agar culture medium at 4 °C. All bacteria strains were sub-cultured on LB agar culture at 37 °C for 24 h.

2.6.2.2. Disc diffusion assay. Antimicrobial properties of chitosan-oil/fat blend films against food-poisoning and human pathogen bacteria were evaluated by disc diffusion assay according to the method [22] with some modifications. Microorganisms' susceptibility was adjusted by 0.5 McFarland as a reference standard. The samples were sterilized under ultraviolet light for 10 min before the assay. Microorganism culture suspension (100 μL, 10^6 cells mL^{-1}) was swabbed onto a plate within Müller-Hinton agar. Filter discs were placed on the Petri plate inoculated with bacteria and then, the tested samples (20 μL) were loaded on the sterile discs. Finally, bacteria cultures were incubated at 37 °C for 24 h. The disc containing gentamicin (10 μg $disc^{-1}$) was used as positive control and disc within only solvent was used as a negative control. All experiments were repeated triplicate. The results were expressed as mean diameter of inhibition zone in mm ± standard deviation (mean ± SD).

2.6.3. Quorum sensing inhibitory activity

2.6.3.1. Disc diffusion test. The reporter strain *Chromobacterium violaceum* CV026 was used to evaluate the quorum sensing inhibitory (QSI) properties of chitosan-oil/fat blend films. For QSI bioassay, *C. violaceum* CV026 bacterial suspensions were sub-cultured by overnight aerobic growth (30 °C) in LB broth. Disc diffusion assay was performed to evaluate the QSI activity of obtained chitosan films according to literature [23] with some modification. Firstly, signal molecules C6-HSL (0.25 μg mL^{-1}) was added to LB soft-agar and then poured into the Petri plates. The strain *C. violaceum* CV026 was inoculated onto a surface of solidified agar. After that, sterile discs were placed onto the agar and loaded with the tested samples (20 μL). Prepared plates were incubated at 30°C for 24–30 h. The disc containing gentamicin (10 μg $disc^{-1}$) and only solvent was used as positive and negative control, respectively. All experiments were repeated triplicate. The QSI activity was determined by the formation of a turbid halo around the disc on a purple background on the plate. The results were expressed as mean diameter of inhibition zone in mm ± standard deviation (mean ± SD).

2.6.3.2. Quantitative determination of violacein inhibition. To determine the quantitative value of violacein inhibition by tested chitosan-oil/fat blend films was carried out by the flask incubation assay. LB broth inoculated with bacteria mixed with C6-HSL (5 μM) and samples was incubated for 24 h at 30 °C. Violacein extraction was carried out according to literature [23]. First of all, for precipitation of violacein, the suspension from each flask (1 mL) was centrifuged at 9.000 *g* for 10 min. For solubilization of violacein, the samples were dissolved in dimethyl sulfoxide (1 mL) and vortexed. Supernatant was centrifuged again and quantified at 585 nm using UV–vis spectrophotometer (PG Instruments, T-60). The assay was done triplicate

and the pigment inhibition percentage was calculated by the formula as:

$$I\% = ((control\ A_{585} - test\ A_{585})/control\ A_{585})X100 \quad (3)$$

3. Results and discussion

3.1. FT-IR spectra analysis

FT-IR spectra of commercial chitosan, olive oil, corn oil, sunflower oil, butter, fat, chitosan-control film and chitosan-oil/fat blend films were recorded to compare the changes in the chitosan film matrix (Fig. 2). As seen from the spectrum of pure chitosan, characteristic bands of chitosan were recorded at 3294.95 cm^{-1} (—OH stretching), 1651.63 cm^{-1} (amide I, C═O), 1554.69 cm^{-1} (amide II, NH_2) and 1314.23 cm^{-1} (amide III, C—N). Aliphatic C—H stretching and C—O bond stretching vibrations were detected at 2874.90 cm^{-1} and 1024.57 cm^{-1}, respectively. With the addition of glycerol and Tween 40, —OH bond stretching vibration was shifted to a higher wavenumber at 3352.51 cm^{-1} after the formation of chitosan film. Aliphatic C–H stretching peaks at the 2917.99 cm^{-1} and 2870.97 cm^{-1} were recorded as sharper after the addition of the Tween 40 and glycerol in the chitosan film spectrum. C═O bond stretching vibration which corresponds to ester structure of Tween 40 was detected at 1736.37 cm^{-1}.

In the spectra of pure plant oils, absorption bands related to olephinic ═C—H stretching were observed in the range of 3005–3008 cm^{-1}. As seen from the spectra, due to the saturated groups of animal fat and butter, for olefinic ═C—H stretching weaker peaks were recorded than those of unsaturated oil samples. The sharp peaks observed in the wave-number range between 2922 and 2853 cm^{-1} were related to the C—H aliphatic stretching vibrations. Since there are saturated fatty acid structures in animal fat and butter, C—H aliphatic stretching vibration peaks were detected sharper. The sharp peak observed at the 1743 cm^{-1} was C═O stretching vibration which correspond to ester structure of fatty acids.

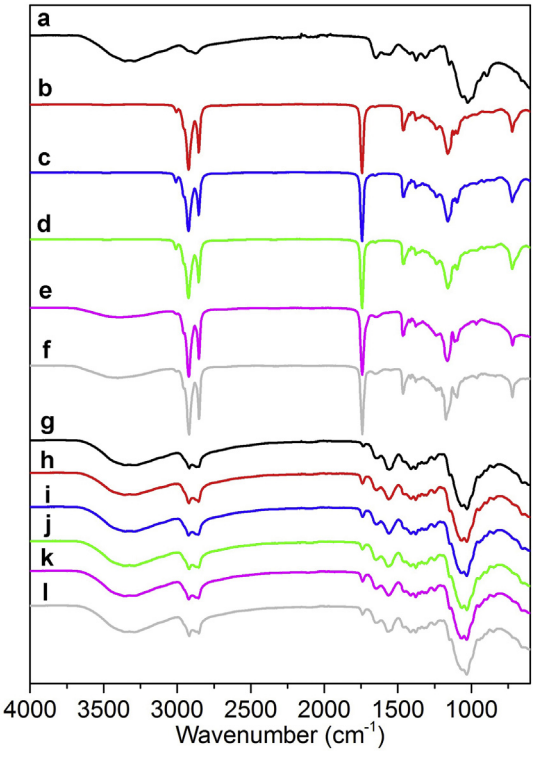


Fig. 2. FT-IR spectra of a) chitosan, b) olive oil, c) corn oil, d) sunflower oil, e) butter, f) animal fat; g) chitosan-control film, h) chitosan-olive oil blend film, i) chitosan-corn oil blend film, j) chitosan-sunflower oil blend film, k) chitosan- butter blend film and l) chitosan- animal fat blend film.

In the spectra of chitosan-oil/fat blend films, the broad absorption bands recorded in the range of 3600–3000 cm^{-1} were attributed to the presence of O—H and N—H groups in chitosan, O—H in glycerol, Tween 40 and adsorbed water. It can be stated that they formed inter- and intra-molecular hydrogen bonding with fatty acids and chitosan. With the addition of the oils into the chitosan film, aliphatic C—H stretching vibration peak intensity increased at 2922–2853 cm^{-1} in the spectra of oil-chitosan blend films. Characteristic peaks of oils (C═O stretching peak at 1743 cm^{-1}) were observed in the spectra of blend films. Also, intensity of amide II peak of chitosan in the blend films was decreased as a result of the possible interactions between chitosan, oil and Tween 40. The changes observed in FT-IR spectra of the film samples with addition of plant oils or animal fat or butter demonstrated the successful incorporation of oils or fatty acids into chitosan film matrix.

3.2. Thermogravimetric analysis of chitosan-oil/fat blend films

TGA was performed to determine the thermal stability of the control (chitosan film) and chitosan-oil/fat blend films. The thermograms are presented in Fig. 3. TGA curves showed a three-stage weight loss for the control, chitosan-olive oil blend film and chitosan-butter blend film. On the other hand, a four-stage of weight loss was observed in the thermograms of chitosan-corn oil blend film, chitosan-sunflower oil blend film and chitosan-fat blend film at temperatures between 100 and 600 °C. In the first stage, similar weight losses resulting from the decomposition of glycerol were recorded for the control, chitosan-olive oil blend film and chitosan-butter blend film at about 150 °C. Weight losses corresponding to the residual water, acetic acid and the other volatile chemicals were observed as 3.58%, 2.97% and 2.77% at about 100 °C for chitosan-fat blend film, chitosan-corn oil blend film and chitosan-sunflower oil blend film. Similar findings were reported by an earlier study by Noshirvani, Ghanbarzadeh, Gardrat, Rezaei, Hashemi, Le Coz and Coma [24]. Two degradation stages were observed for the control, chitosan-olive oil blend film and chitosan-butter blend film in the temperature range of 150–500 °C. However, three degradation steps were recorded for chitosan-fat blend film, chitosan-corn oil blend film and chitosan-sunflower oil blend film in the same temperature range. Degradation observed at around 160 °C was due to the decomposition of glycerol in the films [24]. The main degradation step corresponding to the degradation of chitosan was observed at 315.1 °C for the control. Addition of olive oil, corn oil and butter to the chitosan film increased the thermal stability of chitosan film. The increase in the thermal stability of the chitosan films could be due to the strong interactions between oil and film matrix. Also, this can be attributed to homogeneous dispersion of olive oil, corn oil and butter within chitosan film matrix as it is clearly seen in the SEM images (Fig. 5b, c and e). However, thermal stability of chitosan film was decreased with the incorporation of the sunflower oil. The last weight loss step for all the film samples occurred at around 477–490 °C as result of the degradation of Tween 40. Degradation temperature of Tween 40 was higher for chitosan-corn oil blend film and chitosan-olive oil blend film. This result could be explained by the potential interactions between oil, film matrix and Tween 40.

3.3. Differential scanning calorimetry of chitosan-oil/fat blend films

Chitosan-oil/fat and chitosan-plasticizer interactions were also investigated by DSC. Fig. 4 shows the DSC thermograms of chitosan films with and without oil or fat. The first endothermic peaks at the negative temperature region were melting peak (T_m) of the unsaturated oils for chitosan-olive oil blend film, chitosan-corn oil blend film and chitosan-sunflower oil blend film. In a previous study also reported that the endothermic peak at −8 °C belonged to the melting point of

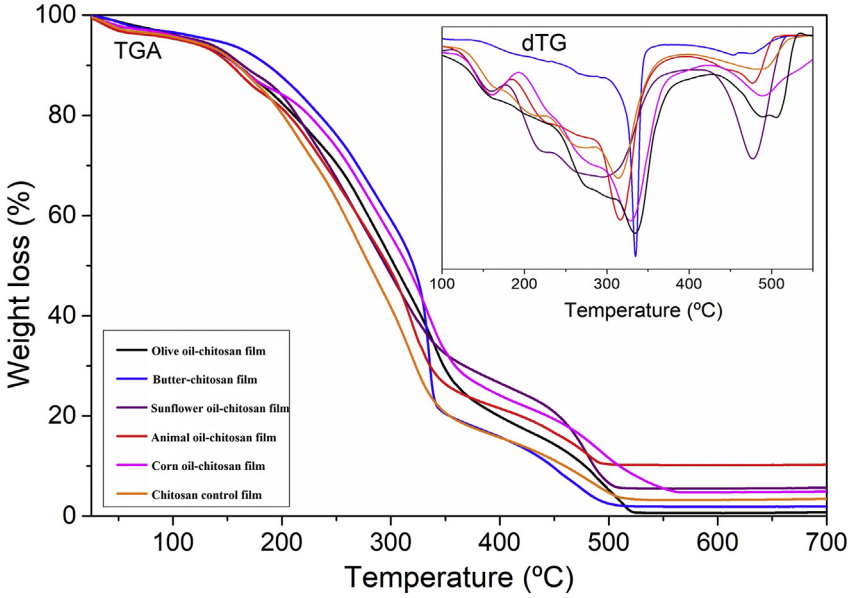


Fig. 3. TGA and DTG thermograms of the chitosan-control film and chitosan-oil/fat blend films a) chitosan-control film b) chitosan-olive oil blend film c) chitosan-corn oil blend film, d) chitosan-sunflower oil blend film, e) chitosan-butter blend film and f) chitosan-animal fat blend film.

olive oil in gelatin-olive oil edible film [25]. As expected, T_m of butter and animal fat was observed in the range of 25–50 °C for chitosan-fat blend film, chitosan-butter blend film and chitosan-sunflower oil blend film. Sharp peaks were observed at the temperature range of 12–15 °C. These peaks can be related to the evaporation of volatile matters found in the film matrix. Evaporation peak of the bounded or non-bounded water was observed as broad peaks at around 50–60 °C.

In some earlier studies, glass transition temperature (T_g) of hydrated chitosan films was reported as 203 °C [26] and 243 °C [27]. However, Madeleine-Perdrillat, Karbowiak, Debeaufort, Delmotte, Vaulot and Champion [28] reported that polysaccharides can be degraded at these temperatures and Dong, Ruan, Wang, Zhao and Bi [29] reported that T_g of hydrated chitosan films were in the range of 140–150 °C. In the present study, T_g of chitosan film (the control) and chitosan-oil/fat blend films was recorded in the range of 128–131 °C. When compared to the reports by Dong, Ruan, Wang, Zhao and Bi [29], this T_g value was relatively lower because of the plasticizer effect of glycerol and oils. T_g value was the lowest (126.59 °C) for chitosan-olive oil blend film while it was the highest for chitosan-butter film (131.68 °C). Different T_g values supported the different structural organization and molecular interactions between oil, fat, glycerol, Tween 40 and chitosan film matrix. T_g value of the chitosan-olive oil blend film was decreased due to the increase of the mobility of the polymer network. This result was in agreement with the elongation at break (Fig. 7). Butter contains 51% saturated, 24% monounsaturated, 23% water and 2% polyunsaturated fatty acids [30]. Palmitic acid with 16 carbons is the most abundant saturated fatty acid found in the butter [30]. The T_g value could be increased by incorporation of butter into the chitosan films and it decreases the mobility of the chitosan matrix due to the relatively shorter chain of the palmitic acid than the other unsaturated and saturated oils with 18 carbons. Crystallization peaks (T_c) of chitosan film (the control) and chitosan-oil/fat blend films were recorded in the temperature range

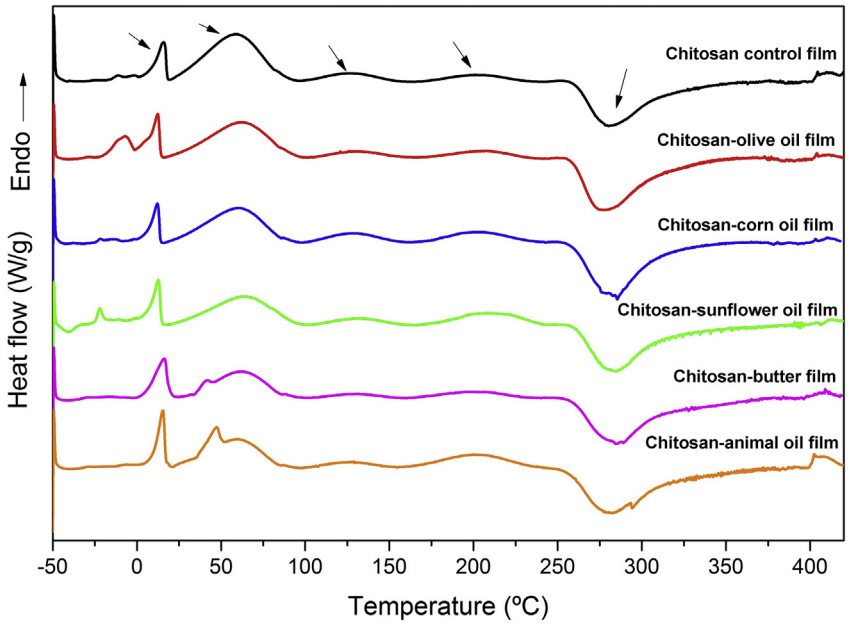


Fig. 4. DSC thermograms of the chitosan-control film and chitosan blend films incorporated with plant oils (olive oil, corn oil, sunflower oil) and animal fats (butter and animal fat).

of 277–280 °C. The lowest and the highest T_c peaks were observed for chitosan-olive oil blend film and chitosan-corn oil blend film, respectively.

3.4. Microstructure of chitosan-oil/fat blend films

The effect of incorporation of oils and fats on the microstructure of chitosan films was shown in Fig. 5. As seen, chitosan control film showed a continuous and nano-fibrous morphology originating from Tween 40. Addition of olive oil formed a continuous and homogenous structure in the polymer matrix. Chitosan-olive oil blend film showed a smooth surface, without any pores or cracks (Fig. 5b). It could be explained by strong molecular interactions between polymer network and olive oil. The presence of olive oil in the film matrix greatly modifies the interactions of the matrix compounds and the film morphology. Incorporation of corn oil and butter into chitosan film network presented the partially similar effect. Some regions of the film surface were smooth and non-porous with no holes. However, aggregation occurred in some on the film surface. As seen from Fig. 5d and f, addition of sunflower oil and animal fat caused discontinuities in the polymer network related to the formation of two phases and flocculation. Oil droplets were observed on chitosan-sunflower oil blend film sample. This is in line with an earlier report by Valenzuela, Abugoch and Tapia [20].

3.5. Transparency of chitosan-oil/fat blend films

Optical properties of packaging materials are important as it affects the quality of the coated commodity. The transparency analysis was carried out spectrophotometrically. The results of spectroscopic scanning at between 400 and 700 nm for the control and chitosan-oil/fat blend films were given in Fig. 6. The values of the transmittance (%) were given in the Table 1. As expected, chitosan-control film had the highest transparency value. Second transparency value was found for chitosan-olive oil film. As seen from Fig. 6, the films formed with unsaturated oils exhibited considerably higher transparent property than the chitosan-saturated oil blend films. Villalobos, Chanona, Hernández, Gutiérrez and Chiralt [31] reported that transparency of the films was greatly affected by the size of the lipids dispersed in the polymer matrix and the initial structure of the dispersion. In the present study, non-consistent transparency properties of the films can be linked to the structure of the oil and size of the chitosan-oil aggregates.

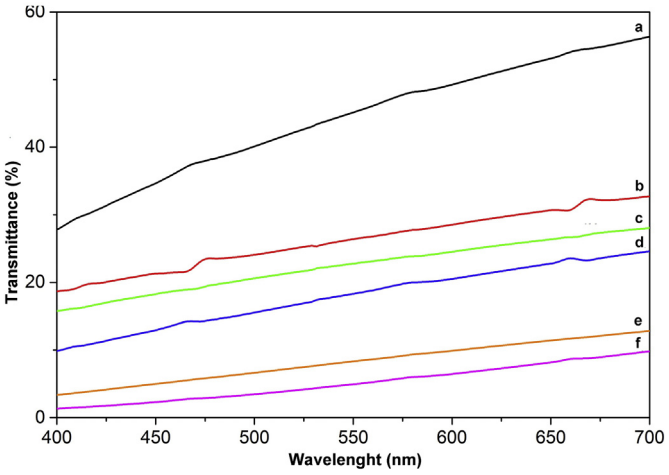


Fig. 6. Optical transmittance of the chitosan-control film and chitosan-oil/fat blend films. a) chitosan-control film and b) chitosan-olive oil blend film c) chitosan-sunflower oil blend film, d) chitosan-corn oil blend film, e) chitosan- animal fat blend film, f) chitosan-butter blend film.

3.6. Contact angle measurements

Surface hydrophobicity of chitosan-oil/fat blend films can be determined by contact angle measurements. The contact angle values with water were recorded as 45.8 ± 1.9, 53.7 ± 4.3, 56.8 ± 2.1, 49.17 ± 2.2, 41.7 ± 2.7 and 46.9 ± 1.6° for the control, chitosan-olive oil, chitosan-corn oil, chitosan-sunflower oil, chitosan-butter and chitosan-animal fat blend films, respectively (Table 1). In a previous study, contact angle of chitosan film containing glycerol and Tween 80 was recorded as 37.3° [17]. In the same study, the contact angle values increased by incorporation of essential oils [17]. Our results were in accordance with results reported by Ojagh, Rezaei, Razavi and Hosseini [17].

Incorporation of olive oil, corn oil, sunflower oil and animal fat into the chitosan matrix increased the contact angles. The highest contact angle value was found as 56.8° for chitosan-corn oil blend film. The

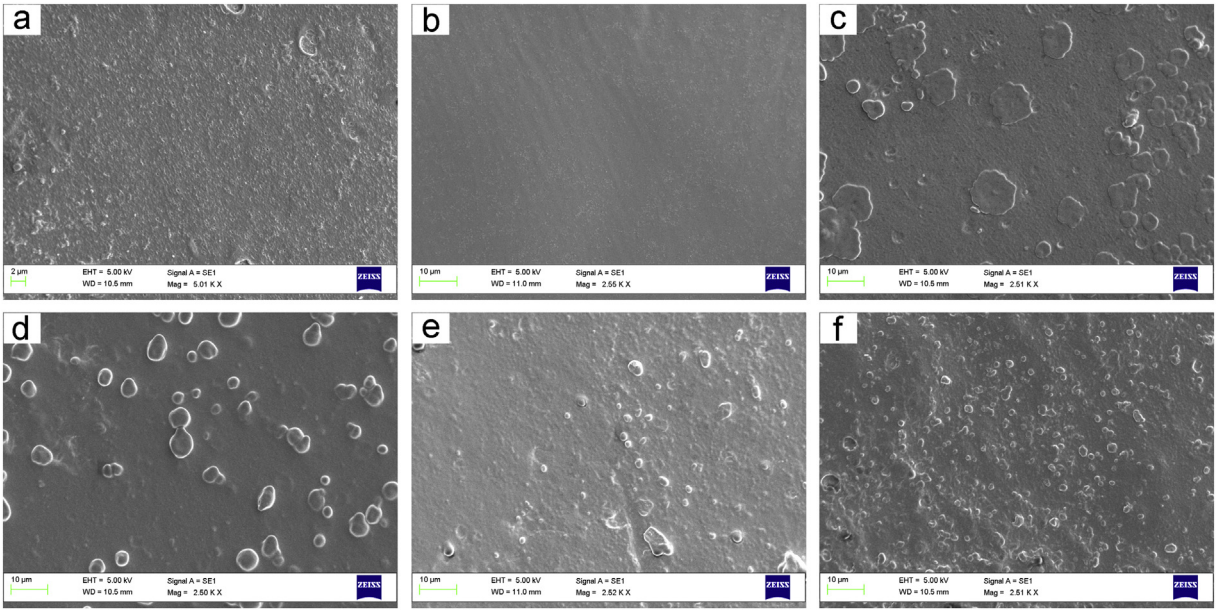


Fig. 5. Scanning electron microscopy of the chitosan-control and chitosan-oil/fat blend films a) chitosan-control film, b) chitosan-olive oil blend film, c) chitosan-corn oil blend film d) chitosan-sunflower oil blend film, e) chitosan-butter blend film and f) chitosan-animal fat blend film.

Table 1
Summary of hydrophobicity, soil degradation, water solubility, antioxidant activity and transparency for chitosan-control and chitosan-oil/fat blend films.

Chitosan-oil/fat blend film	Contact angle (°)	Soil degradation (%)	Water solubility (%)	Antioxidant activity (FRAP) (mg TE g^{-1} film)	Optical transmittance (%)
Chitosan-control	45,8 ± 1,9	85,4	58.02 ± 3.81	19.05 ± 2.30	28–57
Chitosan-olive oil	53,7 ± 4,3	76,02	45.95 ± 1.06	22.24 ± 1.36	19–28
Chitosan-corn oil	56,8 ± 2,1	75,4	53.41 ± 2.11	19.13 ± 0.21	10–18
Chitosan-sunflower oil	49,17 ± 2,2	66,7	53.46 ± 0.15	23.39 ± 0.16	17–22
Chitosan-butter	41,7 ± 2,7	67,9	60.30 ± 1.34	12.64 ± 0.16	1–7
Chitosan-animal fat	46,9 ± 1,6	89,7	56.08 ± 2.12	13.13 ± 1.26	3–9

increase in the contact angle value for chitosan-olive oil film could result from hydrophobic feature of olive oil. Besides, there is a strong interaction between olive oil, Tween 40 and chitosan matrix. The water solubility study showed that chitosan-olive oil film was the least water soluble film. Unexpected decrease in the contact angle of chitosan-butter blend film can be attributed to the remaining butter and Tween 40 on the surface. Hydrophilic groups of the oil and Tween 40 such as carboxyl, hydroxyl were interacted with the water on the film surface and reduced the contact angle.

3.7. Mechanical properties of chitosan-oil/fat blend films

The effect of oils and fats (olive, corn, sunflower, butter and animal oils) on mechanical properties of chitosan films was investigated and shown in Fig. 7. Tensile strength, elongation at break and Young's Modulus values of the chitosan control and chitosan-oil/fat blend films were given in the Table 2. Tensile strength of chitosan-control film was found as 5.54 MPa. Incorporation of olive oil and corn oil into chitosan film increased the tensile strength. Especially chitosan-olive oil blend film exhibited the highest tensile strength. Ojagh, Rezaei, Razavi and Hosseini [17] reported that, if there are strong interactions between oil and the polymer matrix, essential oil can produce a cross-linker effect. So, the molecular mobility of the polymer decreases by increasing the overall tensile strength. The results for chitosan-corn oil and chitosan-sunflower oil films were recorded in agreement with the reports of the study by Ojagh, Rezaei, Razavi and Hosseini [17]. However, both elongation at break and tensile strength values of chitosan-olive oil film were improved when compared to those of chitosan control film. This may be due to the chain length of the olive oil having the highest rate of monounsaturated oil (oleic acid). The higher single bond ratio in long chain molecules, the greater the flexibility and extensibility. When olive oil acted as a cross-linker, the tensile strength was increased by strong interactions between olive oil and chitosan. Because of the monounsaturated nature, long chain of the olive oil caused conformational flexibility in the polymer network and as a result, the elongation at break value enhanced. Pereda, Amica and Marcovich [19] found the same behavior for olive oil-chitosan emulsion films. They explained that enhancement of all the mechanical properties is related to the deacetylation degree and molecular weight of chitosan used [19]. In the current study, it was explained that the increase in mechanical properties is not only dependent on the deacetylation degree and molecular weight of chitosan but also on the chain structure of the additive. Vargas, Albors, Chiralt and González-Martínez [32] also observed that, when the 1% oleic acid incorporated into chitosan films, elastic modulus and tensile strength significantly increased. However, the addition of the oleic acid higher than the 1% caused the decrease in the elastic modulus and tensile strength in the study by Vargas, Albors, Chiralt and González-Martínez [32]. Valenzuela, Abugoch and Tapia [20] reported that the tensile strength values decreased with the increase of sunflower oil concentrations incorporated. The addition of the sunflower oil into the quina protein-chitosan film matrix increased the extensibility. However, the higher concentrations of sunflower oil decreased the extensibility [20]. In the present study, for the chitosan-sunflower oil film, the elongation at break value reduced compared with the chitosan-control film as reported by Valenzuela, Abugoch and Tapia [20]. It is seen from Fig. 5d that the amount of added sunflower oil is excessive. Additionally, elongation values of the chitosan-butter and chitosan-animal oil films enhanced with the effect of the saturated

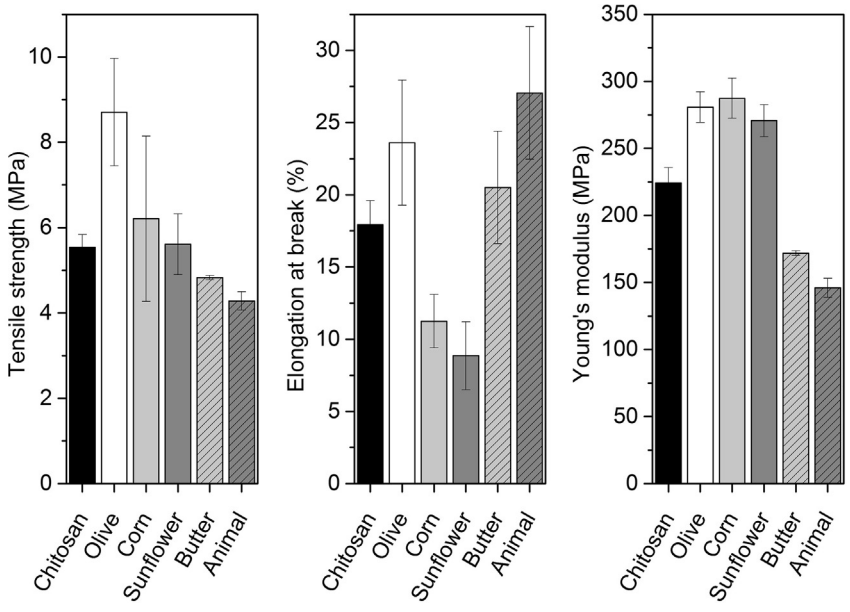


Fig. 7. The mechanical properties of the chitosan-control film and chitosan-oil/fat blend films.

Table 2
Tensile properties of chitosan-control and chitosan-oil/fat blend films.

Chitosan-oil/fat blend film	Tensile Stregth (Mpa)	Elongation at Break (%)	Young's Modulus (Mpa)
Chitosan-control	5.54 ± 0.30	17.92 ± 1.68	224.28 ± 11.45
Chitosan-olive oil	8.71 ± 1.26	23.61 ± 4.33	280.69 ± 11.41
Chitosan-corn oil	6.21 ± 1.94	11.25 ± 1.85	287.36 ± 15.01
Chitosan-sunflower oil	5.61 ± 0.71	8.86 ± 2.35	270.71 ± 11.93
Chitosan-butter	4.83 ± 0.05	20.51 ± 3.90	171.82 ± 1.73
Chitosan-animal fat	4.28 ± 0.22	27.06 ± 4.60	146.00 ± 7.07

fatty acids without double bonds. The double bonds of the molecules have decreased the flexibility and extensibility. According to this observation, in the chitosan-corn oil and chitosan-sunflower oil films which contain a high rate of the polyunsaturated fatty acids and elongation at break values reduced. In the present study, effects of the unsaturated and saturated oils incorporated into the chitosan films on the mechanical properties were clearly determined.

3.8. Water solubility of chitosan-oil/fat blend films

The water solubility property is important in application of edible films as a packaging material. Film solubility in water is related to the biodegradability on the surface of the coated product. The results were given in Table 1. The addition of the oils (olive, corn, sunflower, butter, animal fats) into the chitosan films changed their affinities for water. Cerqueira, Souza, Teixeira and Vicente [18] studied the effect of the glycerol and the corn oil on the chitosan and galactomannon films. It was found that affinity of the polysaccharides films for water decreased with the increase of glycerol and corn oil concentration.

Water solubility of chitosan-olive oil blend film was determined as 45.95% (the lowest value). This can be explained by compact and continuous structure of the chitosan-olive oil film, as seen from the SEM analysis results (Fig. 5b). Also, due to the strong interactions between the carboxylate groups of olive oil and amino groups of chitosan, water molecules cannot reach to the amino groups of chitosan chains. This result was supported in a previous study [19]. Incorporation of butter into chitosan film increased water solubility. This result was in agreement with result of water contact angle for chitosan-butter film.

3.9. Soil degradation of chitosan-oil/fat blend films

Microorganisms that produce enzymes in the soil cause the degradation of the edible films [33]. The soil degradation experiment was carried out up to 15 days to determine the % degradation of the films in the soil. The obtained results were given in Table 1. All of the samples including control and chitosan-oil/fat blend films were degraded in the range of 66.7–89.7% in 15 days. After this time, blend films could not be analyzed. As a result, it can be said that chitosan-oil blend films can be quickly degraded materials in the nature.

3.10. Antioxidant activity of chitosan-control and chitosan-oil blend films

Antioxidant activities of chitosan films incorporated with saturated (butter and animal fat) and unsaturated (olive, corn and sunflower oil) were determined using FRAP method (Table 1). The antioxidant activities were given as equivalent to troloxane, a standard substance. The antioxidant activity of chitosan-control film was determined to be 19.05 ± 2.30 mg TE g^{-1} film. The antioxidant activities of chitosan-corn oil, chitosan-olive oil and chitosan-sunflower oil blend films with high unsaturated contents of the oils were found as 22.24 ± 1.36 mg TE g^{-1} film, 19.13 ± 0.21 mg TE g^{-1} film and 23.39 ± 0.16 mg TE g^{-1} film, respectively. It was determined as 12.64 ± 0.16 mg TE g^{-1} film and 13.13 ± 1.26 mg TE g^{-1} film for chitosan blend films incorporated with saturated oils (butter and animal oil), respectively.

The results showed that antioxidant activity of chitosan films blended increased with addition of sunflower oil. Addition of unsaturated oils to chitosan films increased the antioxidant activity. But, antioxidant activity was decreased with addition of the saturated oils into the chitosan film. Since the antioxidant activities of each fatty acid are different, it can be said that the different fatty acid composition of oil sample can produce this difference. Unsaturated fatty acids, particularly omega 3 unsaturated fatty acids, compared with stearic acid (C18: 0), a saturated fatty acid, it was reported that they had an increasing activity on inhibition of free radical elimination and free radical production [34].

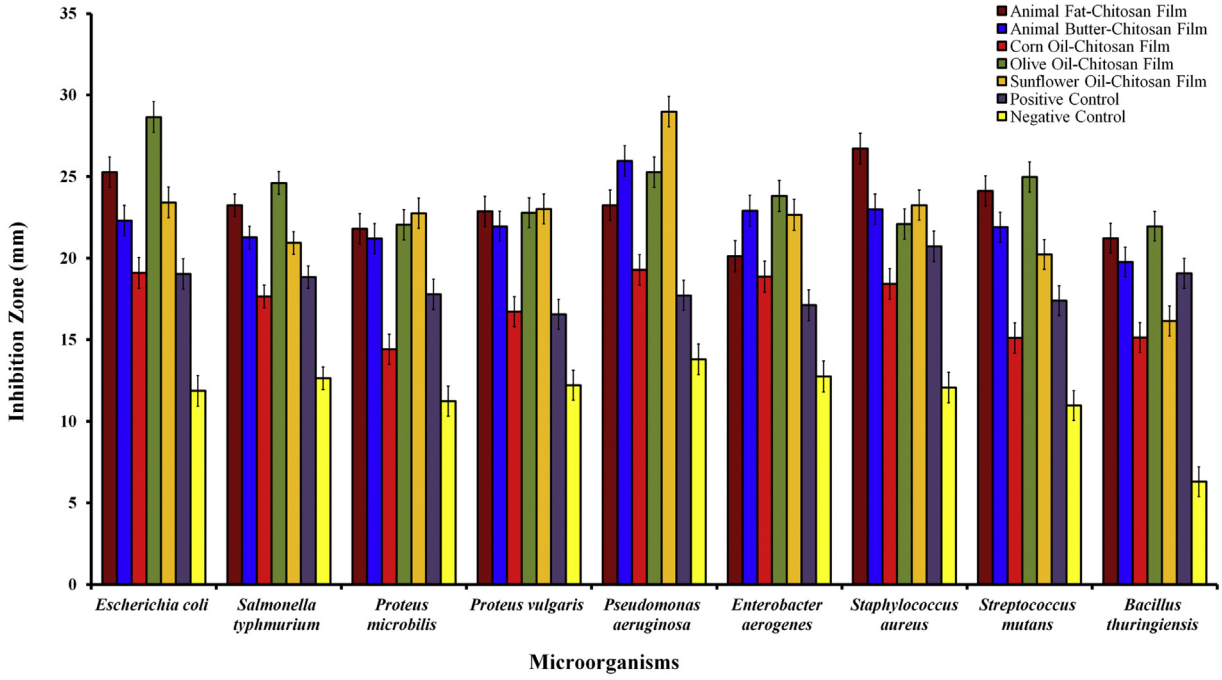


Fig. 8. Antimicrobial activities of different animal fats (animal fat and butter) and plant oils (olive oil, corn oil and sunflower oil) blended chitosan films expressed as inhibition zone diameter (mm).

There is a link between the general composition of animal fats and their living habitat. In sheep fed to different diets, the saturated fatty acids of the animal fat ranged from 38.82% to 47.49% and the omega-3 fatty acids ranged from 0.33% to 0.48% [35]. It is also stated that the total saturation rate of the butter produced from the milk of animals fed with different regimens is 44.97–52.49% and that of omega-3 fatty acids is 0.11% [36]. When compared to butter and animal fat, the rate of saturation of the animal fat is lower while the omega 3 ratio is higher than butter. This can explain the difference between antioxidant activities. There are many cultured species of sunflower in the world. Although the composition of fatty acids in the sunflower oil changes with the different types of culture, it generally comprises approximately 85–90% of the composition of oleic acid (C18: 1) and linoleic (C18: 2) acids [37]. Similarly, about 85% of the overall composition of corn oil is composed of oleic (C18: 1) and linoleic (C18: 2) acids [38]. 73.6–81.6% of the general composition of the olive oils produced in our country is composed of oleic acid (C18: 1 n9) [39]. However, antioxidant activity is expected to be high due to the fact that it contains some phenolic substances.

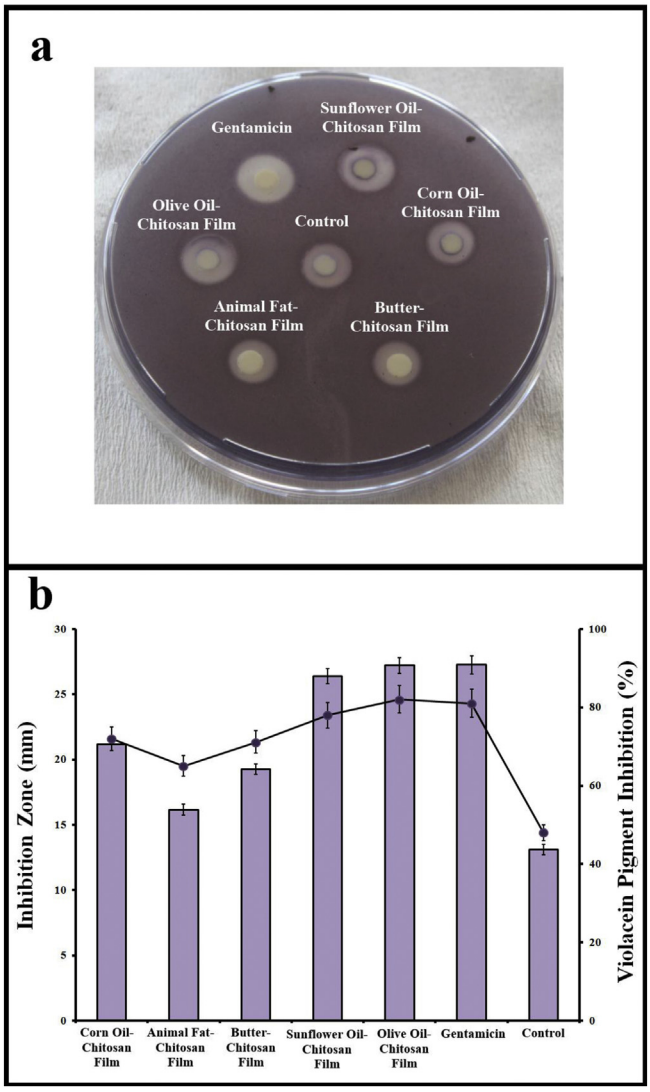


Fig. 9. (a) Plate diffusion assay for the quorum sensing inhibition. (b) Inhibition zone (mm) (*purple bars*) of disc diffusion test and quantitative determination of violacein inhibition (%) (*purple squares*) of different animal fats (animal fat and butter) and plant oils (olive oil, corn oil and sunflower oil) blended chitosan films.

3.11. Antimicrobial properties of chitosan-oil/fat blend films

Antimicrobial activity of chitosan-oil/fat blend films was investigated against food-borne and human pathogen bacteria. Mean inhibition values for antimicrobial properties chitosan-oil/fat blend films are presented in Fig. 8. It is interesting that antimicrobial activity of all tested chitosan films was greater than negative control (only solvent). Plant oils and animal fats blended chitosan films were strongly effective against all of the tested bacteria strains with mean inhibition zone values range of 14.56 ± 0.81 mm (*P. microbilis*) to 29.84 ± 0.92 mm (*P. aeruginosa*) as their inhibition zone diameter increased about 45.0 ± 2.2% against tested pathogens compared with negative control. This might be corresponded to the entrapment of oil compounds into the chitosan film improves the stability of film mechanical properties which leading to efficient activity of antimicrobial compounds against pathogens. In all test, control (only solvent) showed inhibition zone ranged with 6.32 ± 0.45 mm 13.81 ± 0.78 mm.

Interestingly, the common antibiotic gentamicin exhibited lower values of inhibition zone (ranged from 16.56 ± 0.62 to 20.73 ± 0.84 mm) against almost of the tested bacteria than chitosan film blended with different oil compounds. Sunflower oil and olive oil incorporated chitosan films displayed higher antibacterial activity than other film samples (corn oil, animal fat and animal butter). These results suggest that chitosan blended with plant oils or animal fats could be used as a new generation, non-toxic and hydrophobic antimicrobial agent. In general, antimicrobial film's functionality in food packaging can be evaluated from the extent of microorganism growth. Chitosan-oil/fat blend films produced in this study are nontoxic, biodegradable and mechanically stable. Thus, they could be used as food packaging material that is capable of protecting both the food content and the environment.

3.12. QSI bioactivity of chitosan-oil/fat blend films

In the present study, chitosan-control and chitosan-oil/fat blend films were assessed for the QSI property against the reporter strain *C. violaceum CV026* (Fig. 9). In the disc diffusion method (Fig. 9a), all of the tested chitosan films displayed high values of QSI activity (from 16.15 ± 0.64 mm to 27.25 ± 0.82 mm) which was evaluated by the loss of purple pigmentation (halo formation around the disc). Regarding to radios of inhibition zone, chitosan films with olive oil and sunflower oil showed higher pigment clearance than other oil incorporated chitosan film and also the pigment inhibition of chitosan based films blended with olive oil (27.21 ± 0.76 mm) showed interestingly almost equal inhibition zone with commercial antibiotic gentamicin (27.26 ± 0.77 mm). On the other hand, quantitative determination of the violacein production inhibition was also exhibited that chitosan films blended with different oil compounds have ability to reduce the production of violacein, which ranged from 65.58 ± 2.9% to 82.12 ± 3.2% (Fig. 9b). Similarly, olive oil and sunflower incorporated chitosan films showed a significant inhibition of violacein production (82.12 ± 3.2% and 78.44 ± 3.1%, respectively) compared with other oil contained chitosan films (Fig. 2b). In this study, for the first time the potential use of animal fat/butter and plant oils blended chitosan films was evaluated as anti-QS agents owing to their ability in inhibiting the violacein production without inhibition of bacteria growth. Chitosan-oil/fat blend films can be used to regulate biological functions, such as the production of signaling molecules, antibiotics, other microbial metabolites, and even virulence [40]. Owing to their high anti-QSI activity of, chitosan-oil/fat blend films may undergo bacterial signaling behavior without allowing bacteria to grow to high density.

4. Conclusions

In the present study, daily consumed plant (olive, corn and sunflower) oils butter and animal fat with different unsaturation degrees and chain structures were incorporated into the chitosan blend films

for production of the low cost biodegradable composite films. As a result, obtained films presented different biological, physicochemical and mechanical properties depending on the chain structures and unsaturation degrees of oils. The antioxidant activities of the chitosan-unsaturated oil blend films were higher than the chitosan-saturated oil films. Plasticizing effect of saturated, monounsaturated and polyunsaturated fatty acids was explained according to conformational flexibility of the chain for the first time. Chitosan-olive oil film with the highest rate of monounsaturated fatty acids showed the best mechanical properties and hydrophobicity than the other chitosan-oil blend films and chitosan-control film. It was determined that chitosan-unsaturated oils films significantly showed the higher transparency property than chitosan-saturated oils control films. The results were showed that chitosan-oil/fat blend films can be used for food packaging and preservation as an alternative to synthetic materials.

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