



## Characterization of Chitosan-Based Films with Different Aniseed Oil Content

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**Abstract.** This paper reports the findings of a study describing the production of chitosan-based edible films incorporated with different amount of *Pimpinella anisum* seed oil. The chitosan-aniseed oil (CAO) composite films (CS, CAO-0.5, CAO-1, and CAO-2) were characterized with FT-IR and DSC and their mechanical, optical transmittance, hydrophobicity and anti-oxidative properties were also investigated. With the increasing concentration of aniseed oil in the films, a continuous decrease was observed in tensile strength and Young's modulus. Optical transmittance of chitosan-aniseed oil composite films in the UV-Visible region was lower than that of chitosan control film (without aniseed oil). Incorporation of aniseed oil into chitosan films enhanced antioxidant activity of films. The results suggested that chitosan-aniseed oil composite films can be used as a food packaging material.

**Keywords:** *Pimpinella anisum*, chitosan film, eco-friendly material.

## Farklı Oranlarda Anason Tohumu Yağı Katkılı Kitosan Temelli Filmlerin Özelliklerinin Belirlenmesi

**Özet.** Bu çalışma ile farklı miktarlarda *Pimpinella anisum* tohumu yağı katkılı kitosan temelli yenilebilir filmlerin üretimi yapılmıştır. Kitosan-anason tohumu yağı (CAO) katkılı kompozit filmler (CS, CAO-0.5, CAO-1 ve CAO-2) FT-IR ve DSC ile karakterize edilmiştir ve mekanik, optik geçirgenlik, hidrofobiklik ve anti-oksidan özellikleri araştırılmıştır. Filmlerin anason tohumu yağı miktarı arttıkça, gerilme mukavemeti ve Young modülünde düşüş gözlenmiştir. UV-Görünür bölgede kitosan-anason tohumu yağ kompozit filmlerin optik geçirgenliği, kitosan kontrol filme göre daha düşük olduğu görülmüştür. Anason tohumu yağının, kitosan filmlere katılması, filmlerin antioksidan aktivitesini arttırmıştır. Sonuçlar göz önüne alındığında, kitosan-anason tohumu yağı katkılı kompozit filmlerin, gıda ambalaj malzemesi olarak kullanılabilir bir malzeme olduğunu göstermektedir.

**Anahtar Kelimeler:** *Pimpinella anisum*, kitosan film, çevre dostu malzeme.

### 1. INTRODUCTION

Due to the adverse effects of petroleum-derived packaging products used in the food industry, researchers are focusing on the production of

packaging materials with natural additives. Many recent studies demonstrated that plant oils exhibits good antioxidant activity against various microorganisms. So, plant oils are now considered an alternative to synthetic packaging materials that

are detrimental to human health [1, 2]. In addition, there is a growing research interest in enhancing the efficiency of composite films prepared with biopolymers such as alginate, cellulose, chitosan and plant oil extracts [3-5].

Chitosan, deacetylated form of chitin, is the most abundant biopolymer after cellulose and it has been widely used in many applications including the food industry. Chitosan is considered to be an ideal biopolymer for production of edible films due to its non-toxicity, biocompatibility, biodegradability, antioxidant, antibacterial and film-forming ability [6]. Due to its amine groups, chitosan is capable of interacting effectively with functional groups of various compounds and thus forming stable composite films [6, 7]. In order to increase the structural, mechanical and antimicrobial properties of chitosan composite films, oil from plants such as garlic, bergamot, cumin, rosemary, cinnamon and thyme has been successfully incorporated into chitosan-based films [8-12]. In the current study, *Pimpinella anisum* seed oil was incorporated into chitosan-based films for the first time to enhance the structural properties of films.

*P. anisum*, commonly known as aniseed, is one of the oldest species used by people, being cultivated first in Egypt and later in Greece, Rome, and the Middle East. Anise has white flowers and yellow-brown or green-brown fruits, which contain not less than 2% (w/w) of essential oil [13]. The traditional uses of aniseed (*Pimpinella anisum* L.) for dyspeptic complaints, namely spasmodic gastrointestinal ailments, bloating and flatulence, and catarrh of the upper respiratory tract are supported by experimental data. The medicinal use of aniseed is largely due to antispasmodic, secretolytic, secretomotor, and antibacterial effects of its essential oil. Pharmacological data show a significant relaxing effect of aniseed alcoholic extracts and essential oil on tracheal and ideal smooth muscles contracted by several contraction-inducing agents (e.g., metacholine and carbachol). Moreover, this indication is also made plausible by the secretolytic and expectorant effects exhibited by anethole, the main component of anise oil. Anise exhibits antimicrobial, anti-mutagenic, and

antipyretic activities. Furthermore, it shows anticonvulsant effects, and also is used for the treatment of constipation [14].

In the present study, chitosan blend films were prepared with different concentrations of *P. anisum* seed oil (0.5, 1 and 2 mL). The structural and physicochemical properties of chitosan-based blend films were investigated using FT-IR, DSC and optical transmittance analysis. Mechanical properties such as tensile strength, young modulus and elongation at the break and antioxidant activities of the films were investigated.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Medium molecular weight chitosan was obtained from SIGMA ALDRICH (448877-50G). The anise seeds used were taken from a local market and all other chemicals were supplied from SIGMA ALDRICH.

#### 2.1.1. Extraction of oil from *P. anisum* seed

Anise seeds were washed with distilled water, dried at room temperature protecting from direct sunlight. Dried seeds were milled with a blender. *P. anisum* seed oil was extracted from 10.0 g of anise seeds in petroleum ether in 24 h. In the purification step the extract was filtered through a Whatman paper and ether was evaporated in an evaporator (Hei-VAP Advantage, Heidolph). In a previous study, the same method was used [15].

#### 2.2. Preparation of chitosan-based aniseed oil films

Four different films were produced in the study. For each film, 20 mg of chitosan was dissolved in 1% acetic acid solution (20 mL) and 4 drops of glycerol were added as plasticizer. One of the film was prepared as a chitosan control film (CS). The others were labelled as CAO-0.5 (Chitosan aniseed oil-0.5mL), CAO-1 (chitosan aniseed oil-1mL) and CAO-2 (chitosan aniseed oil-2mL) by adding 0.5, 1.0 and 2.0 mL anise oil, respectively. The films containing glycerol and various proportions of

anise seed oil were homogenized at 26.000 rpm for 10 minutes with a homogenizer (Heidolph, SilentCrusher M). The samples were poured into Petri dishes and allowed to dry at 30°C for 48 h. The films were studied using a modified version of previous study reported by [15, 16].

## 2.3. Physicochemical analysis

### 2.3.1. Film thickness

Thickness of the films was measured on a Coolant Proof Micrometer-293 Mitutoyo USA. For each film sample, more than 10 measurements were done and average values were calculated.

### 2.3.2. Attenuated total reflectance infrared spectroscopy (ATR/ FT-IR)

The infrared spectra of the films were recorded with Perkin-Elmer FT-IR spectrometer at 4000-650  $\text{cm}^{-1}$ .

### 2.3.3. Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) for the films was conducted on Mettler Toledo DSC822e (Switzerland) system. DSC thermograms of the films were obtained under  $\text{N}_2$  atmosphere. The samples were placed in a hermetic aluminium pan and then heated from -50°C to 420°C at a heating rate of 5°C. The changes were recorded.

### 2.3.4. Mechanical properties

The films with dimensions of 5 x 40  $\text{mm}^2$  were cut out for analysis. Tensile tests were performed using a Material Testing Systems (MTS Insight 10) instrument with a load cell of 250 N and a deformation rate of 5 mm/min. The tensile strength, Young's modulus and tensile-elongation tests were determined using the MTS Test Works 4 software.

### 2.3.5. Optical transmittance

Optical properties of the films were studied on a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer in 400–700 nm.

### 2.3.6. Contact angle measurements

The sessile drop contact angle of the films was done by using a Data Physics video-based contact angle measurement system OCA20. Precise drop volume was measured using software controlled dosing volume weight-drop. For surface energy measurements water is used. The measurements were repeated for ten times for each film sample.

## 2.4. Biological properties

### 2.4.1. Antioxidant activity

Antioxidant activities of the films were determined by using DPPH radical scavenging method. Accordingly, small pieces of composite film samples weighing 10 mg were cut out and placed in the test tubes as 3 replicates for each sample. DPPH solution at a concentration of  $6 \times 10^{-5}$  M was added onto these samples. Then the mixture was rested in the dark at room temperature for 3 h. At the end of the period the absorbance of the mixture was measured at 517 nm and the inhibition rates of the samples were calculated according to the following equation;

$$\text{Inhibition (I\%)} = ((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100$$

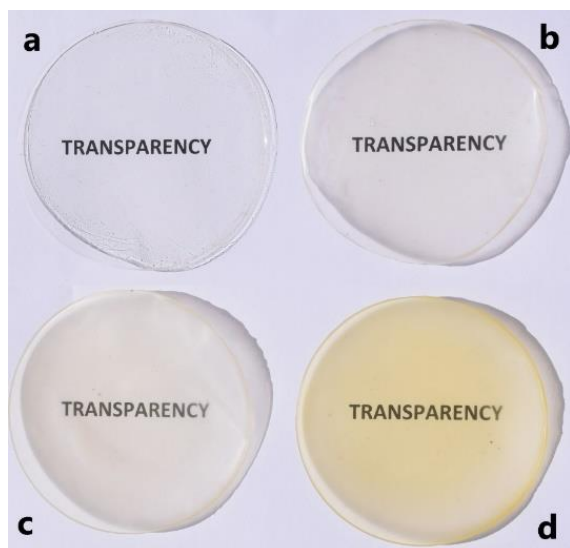
### 2.4.2. Fatty acid composition analysis

Oil sample obtained from anise seeds was esterified according to IUPAC (1979). Analyses were conducted using HP Agilent 6890N Gas Chromatography and HP-88 column. For identification of fatty acids, retention time values for fatty acid standards (Alltech and Accu Standard) were evaluated.

## 3. RESULT AND DISCUSSIONS

### 3.1. Thickness of the films

Transparency of the films is depicted in Fig. 1. The thickness of each film sample was recorded as follows;  $24.2 \pm 3.6$  mm for CS,  $48 \pm 7.7$  for CAO-0.5,  $60.3 \pm 3.6$  mm for CAO-1 and  $94 \pm 8.2$  for CAO-2 mm.



**Figure 1.** Images of (a) CS, (b) CAO-0.5, (c) CAO-1 and (d) CAO-2 films.

### 3.2. ATR/ FT-IR

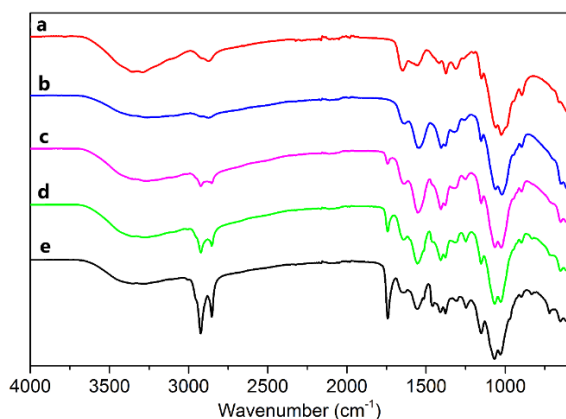
FT-IR spectra of pure anise seed oil, CS and anise seed oil (AO) added chitosan films were obtained in order to explain the interactions between chitosan and AO. The spectra were recorded in the spectral range of the 650–4000  $\text{cm}^{-1}$  and it is presented in Fig. 2.

The peaks in the spectrum of pure anise seed oil (Fig. 2) confirmed the presence of the fatty acid compositions analysis. The sharp peaks corresponding to the C-H aliphatic stretching vibrations of fatty acid esters were recorded at 2923.6  $\text{cm}^{-1}$  and 2853.7  $\text{cm}^{-1}$ . Carbonyl group peaks of ester structures were observed at 1743.5  $\text{cm}^{-1}$ . C=C stretching vibrations of unsaturated fatty acid esters structures appeared at 1608.7  $\text{cm}^{-1}$  and 1510.4  $\text{cm}^{-1}$ . The peaks recorded at 1464.4  $\text{cm}^{-1}$  and 1377.2  $\text{cm}^{-1}$  was due to C-H bending and  $-\text{CH}_3$  group bending of aliphatic esters, respectively. The stretching vibration peaks of the C-O-C linkage which is the characteristic peaks of the ester structures appeared at 1245.5  $\text{cm}^{-1}$  and 1173.9  $\text{cm}^{-1}$ . Three peaks observed in the range of 1037.7  $\text{cm}^{-1}$  - 838.23  $\text{cm}^{-1}$  can be ascribed to out-of-plane bending of the =C-H bond of unsaturated fatty acid esters.

The spectra of obtained films were compared with each other. As is seen from the spectrum of CS film

(Fig. 2) without anise oil, when the acetic acid was added to chitosan,  $-\text{OH}$  and  $-\text{NH}$  bands of chitosan appeared at 3290  $\text{cm}^{-1}$  and 3295  $\text{cm}^{-1}$  and broadened C-H aliphatic stretching peak of chitosan. This result showed that hydrogen bridges occurred among water, acetic acid and chitosan in chitosan film. Amide I band of chitosan was recorded at 1633.2  $\text{cm}^{-1}$ . Amide II and amide III bands of chitosan were sharpened and shifted to lower wavenumbers. These results can indicate that new hydrogen bands found between  $\text{NH}_2$  groups of chitosan and  $-\text{OH}$  groups of acetic acid.

An informative way to interpret the spectra of the films incorporated with AO is to make a comparison to CS film spectrum. The differences between CS film and CAO films can be interpreted in terms of the structural differences. With the addition of AO into to chitosan film, aliphatic C-H stretching vibration peak (2870.6  $\text{cm}^{-1}$ ) was cleaved into two peaks at the CAO films spectra by affecting of aliphatic C-H bonds of anise. Aliphatic CH stretching vibration intensity of CAO-0.5 and CAO-1 gradually increased with an increase in the amount of AO. However, the intensity of aliphatic CH stretching vibration peak in the spectrum of CAO-2 film was lower than those in the spectra of CAO-0.5 and CAO-1. This result can be attributed to more hydrogen bonding interactions in CAO-2 film. As a result, hydrogen bond peak was enlarged and the peak intensity of C-H stretching was decreased. C=C aliphatic stretching vibration peak of AO was not observed in the spectra of CAO-0.5 and CAO-1, while it was observed at 1511.2  $\text{cm}^{-1}$  for CAO-2. Also, the out-of-plane bending of =C-H bond of unsaturated fatty acid esters appeared at 962.61  $\text{cm}^{-1}$  and 837.62  $\text{cm}^{-1}$  in the spectrum of CAO-2. The differences in FT-IR analysis results of the films showed that AO was successfully incorporated into chitosan films.



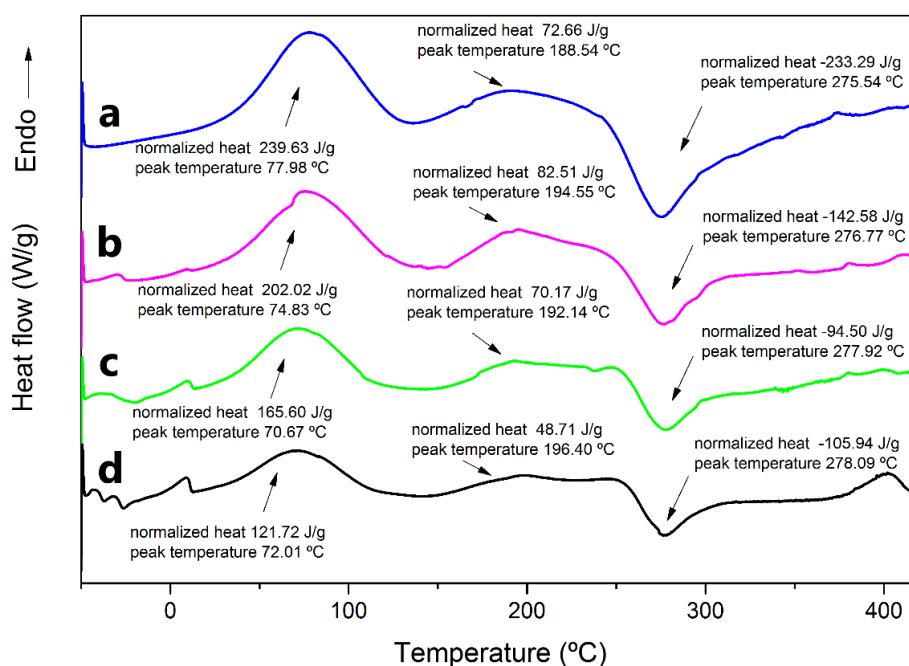
**Figure 2.** FT-IR spectra of; (a) chitosan, (b) CS, (c) CAO-0.5, (d) CAO-1 and (e) CAO-2 films.

### 3.3. DSC

DSC was used to investigate the interactions of chitosan with anise seed oil and glycerol. DSC curves of CS, CAO-0.5, CAO-1 and CAO-2 films

were presented in Fig. 3. Two endothermic and exothermic peaks were recorded for all the films. The first endothermic peak observed at around 77-90°C can correspond to the evaporation of bounded or non-bounded water molecules and to volatile compounds found in the film matrix.

Different  $T_g$  values for chitosan films were reported as 203, 243 and 140°C in earlier studies [17-19]. In the current study, the second endothermic peak at around 188-194°C corresponds to the  $T_g$  values of CS, CAO-0.5, CAO-1 and CAO-2 films.  $T_g$  value was the lowest (188.54°C) for chitosan control film while it was the highest (196.40°C) for CAO-2 film. The exothermic peak observed at nearly 275-278°C corresponds to the crystallization peak of chitosan blend films. The lowest  $T_c$  was obtained for the chitosan control and the highest  $T_c$  was for CAO-2 film.



**Figure 3.** DSC thermograms of; (a) CS, (b) CAO-0.5, (c) CAO-1 and (d) CAO-2 films.

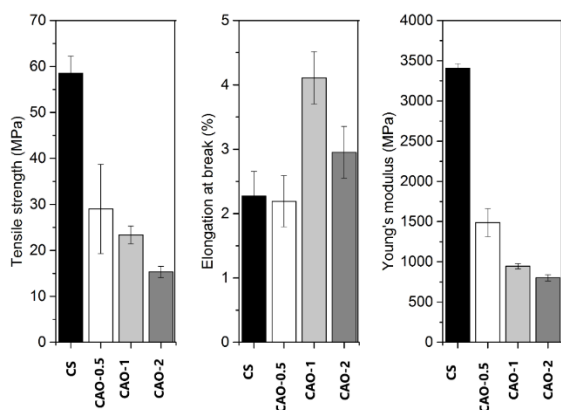
### 3.4. Mechanical properties

The mechanical properties of chitosan blend films were examined to investigate the changes in the molecular interactions of film matrix. As seen from Fig. 4, incorporation of aniseed oil into chitosan film altered the mechanical properties of chitosan

blend films. Compared to the chitosan control film, tensile strength (TS) and Young's modulus (YM) values of chitosan-aniseed oil blend films significantly decreased. This reduction in TS and YM of chitosan-aniseed oil blend films can be attributed to the structural rearrangements in the film matrix. In our earlier study, a decline in TS and

YM values were reported when capsaicin was incorporated into chitosan film [20]. Similarly, these results were in accordance with Hosseini et al study [12].

A significant increase in Elongation break (EB) was observed when 1.0 mg of aniseed oil was incorporated into the chitosan films. This result can be attributed to the increase in the flexibility caused by polymeric chain mobility. Similar findings were also observed in a previous study [21]. However, EB value of chitosan blend film decreased with the increasing aniseed oil extract concentration. This can be due to the changes in intra and intermolecular interactions between fatty acids and chitosan found in the film matrix.

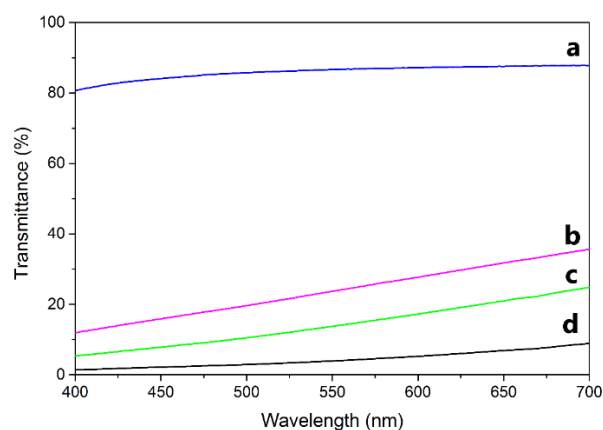


**Figure 4.** Mechanical properties of; (a) CS, (b) CAO-0.5, (c) CAO-1 and (d) CAO-2 films.

### 3.5. Optical Transmittance

The transparency properties of CS, CAO-0.5, CAO-1 and CAO-2 films are shown in Fig.1. Film transparency is important since coating materials in the packaging industry can directly affect the oxidation rate of lipids and thus can damage food quality [22, 23]. The transmittance of the prepared films (CS, CAO-0.5, CAO-1 and CAO-2) was examined between 400-700 nm wavelengths. The changes in the films depending on the amount of anise seed oil are shown in Fig.5. Optical transmittances of CS, CAO-0.5, CAO-1 and CAO-2 films were determined as follows; 80-87%, 11-35%, 5-24% and 1-8% (Table 1). In a previous study, chitosan composite films were formed by adding *Camelina sativa* seed oil. It was found that

the addition of *C. sativa* seed oil decreased the transmittance of chitosan films [15]. This reduction in transmittance may be beneficial in protecting food; because ultraviolet light is obviously the most harmful effect of light on food [11]. In the present study, the addition of anise seed oil into chitosan decreased the transmittance of CS film. These results suggest that chitosan films containing anise seed oil may potentially delay lipid oxidation in food products, which is caused by UV-visible light.



**Figure 5.** Optical transmittance of; (a) CS, (b) CAO-0.5, (c) CAO-1 and (d) CAO-2 films.

### 3.6. Contact angle

The contact angles of the films were recorded at  $65.4 \pm 1.87^\circ$  for CS,  $76.9 \pm 3.3^\circ$  for CAO-0.5,  $82.8 \pm 2.2^\circ$  for CAO-1 and  $82, 1 \pm 6.2^\circ$  for CAO-2. As known chitosan has a hydrophilic structure but the addition of hydrophobic anise seed oil increased the hydrophobicity of the films with relation to amounts of aniseed oil. The results showed that as the amount of fat increased, the contact angle increased. These results are similar to the results of a previous study with chitosan-*C. sativa* seed oil composite films [15].

### 3.7. Antioxidant activity

Antioxidant activity of the films was studied by following the DPPH radical scavenging activity method. As expected, the total phenol content of chitosan film increased significantly after the addition of anise seed oil. Chitosan control film showed 72.74% inhibition. Antioxidant activity was also increased by the addition of anise seed oil into chitosan films. Increasing anise seed oil content of the films increased their antioxidant activity; CAO-0.5 (75.65%), CAO-1 (82.16%) and

CAO-2 (82.20%). CAO-1 and CAO-2 films exhibited antioxidant activity similar to each other (Table 1). This can be attributed to the constant concentration of free radicals despite an increase in unsaturated fatty acid in the medium. Anise seed

oil has a rich content of petroselinic acid, which is used as moisturizing, skin-irritation reducing agent and anti-aging agents in cosmetic products [24]. Antioxidant activity is closely related to anti-aging and skin-irritation reducing property.

**Table 1.** Antioxidant activity an physicochemical properties of CS, CAO-0.5, CAO-1 and CAO-2 films.

	Antioxidant activity	Optical transmittance	Tensile strength (Mpa)	Elongation at break (%)	Young Modulus (Mpa)
<b>CS</b>	72.74±0.87	%80-87	58.52 ±3.71	2.27 ±0.39	3405.73 ±57.10
<b>CAO-0.5</b>	75.65±1.23	%11-35	29.02 ±9,73	2.19 ±0.40	1487.84 ±175.46
<b>CAO-1</b>	82.16±0.23	%5-24	23.35 ±1.93	4.11 ±0,40	945.68 ±32.00
<b>CAO-2</b>	82.20±0.46	%1-8	15.31 ±1.24	2.95 ±0.4	801.72 ±37.71

### 3.7. Fatty acid composition analysis

Anise seed oil consists mainly of myristic acid, linoleic acid and petroselinic acid (Table 2).

Petroselinic acid is a characteristic fatty acid of the Apiaceae family. Anise (*P. anisum* L.) is a member of this family and it has a fatty acid content of 57.78%.

**Table 2.** Fatty acid compositions (%) of *P. anisum* L. seed oil.

Fatty Acids	Mean±SD	Fatty Acids	Mean±SD
<b>C 12:0</b>	0.35±0.03	<b>C 18:1 n9</b>	1.20±0.01
<b>C 13:0</b>	0.04±0.01	<b>C 20:1 n9</b>	0.15±0.01
<b>C 14:0</b>	17.10±0.57	<b>ΣMUFA</b>	<b>59.42±0.43</b>
<b>C 15:0</b>	0.04±0.01	<b>C 18:2 ω6</b>	18.16±0.10
<b>C 16:0</b>	3.36±0.05	<b>C 18:3n6</b>	0.15±0.01
<b>C 18:0</b>	1.00±0.01	<b>C 18:3n3</b>	0.38±0.01
<b>C 20:0</b>	0.01±0.01	<b>ΣPUFA</b>	<b>18.69±0.11</b>
<b>ΣSFA</b>	<b>21.90±0.54</b>	<b>ΣUFA</b>	<b>78.11±0.54</b>
<b>C 16:1 ω7</b>	0.29±0.01	<b>EFA</b>	<b>18.54±0.11</b>
<b>C 18:1 n6</b>	57.78±0.43	<b>Total</b>	100.01±0.01

SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; UFA: Unsaturated fatty acids; EFA: Essential fatty acids., IUPAC 1979. Standards Methods for Analysis of Oils, Fats and Derivatives, (C. Paquot, ed.) pp. 59–66, Pergamon Press, Oxford, U.K.

## 4. CONCLUSION

*P. anisum* L. seed oil was extracted and then successfully incorporated into chitosan films. Chitosan-based anise seed oil films were characterized thermally, optically and mechanically. The antioxidant activity of the films was also studied. It was observed that anise seed oil, which is one of the best natural antioxidants, greatly improved the antioxidant activity of the films. FT-IR spectra analysis demonstrated that functional groups of hydrophobic anise seed oil molecules interacted with the functional group of hydrophilic chitosan. Addition of *P. anisum* seed oil into chitosan matrix made chitosan films more flexible when compared to chitosan film without any anise seed oil. This enhancement in the

flexibility of the films can be ascribed to the petroselinic acid content of aniseed oil. Furthermore, aniseed oil molecules improved hydrophobicity of the films but it reduced the transparency of the films. It appears that these properties make chitosan- *P. anisum* seed oil composite films a suitable material for food packaging industry.

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