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The Antioxidant Properties Of Chitosan Encapsulated With The Oleuropein Extracted From Olive Leaf

Gülay Baysal[*1]
[1]*Nutrition and Dietetic Department, Faculty of Health Sciences, Istanbul Aydin University, Istanbul, 34295, Turkey*
**Corresponding Author: gulaybaysal@aydin.edu.tr*

Abstract
In this study, the chitosan, a polysaccharide, was encapsulated with oleuroperin. Freeze-drying method was used in the encapsulation process. The freeze cooling temperature was determined as -80 ^{o}C. The obtained encapsulated chitosan compounds were analyzed by Fourier transform infrared spectroscopy (FTIR) and their molecular weights were determined by the cryoscopy method. The total amount of phenol compounds and the % antioxidant activity of the synthesized compounds were measured by UV spectrophotometer. According to the results of the analysis, the antioxidant activity values of %66.22. Molecular weights were calculated as 67 and 88 kDa for chitosan (CS) and CSx compounds, respectively.

Keywords: *Encapsulation, the oleuropein, chitosan, antioxidant properties*

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I. INTODUCTİON

The olive leaves have therapeutic effects against heart attack, rhythm disturbance, vascular occlusion, cancer, hypertension, diabetes mellitus and hypoglycemia, and because of their high anti-microbial properties, researchers started to investigate use of olive leaves against many viral diseases, particularly HIV (Bouaziz et al., 2008; Barbaro et al., 2014). They are also used to cure neurological disorders including Parkinson's and Alzheimer's diseases. The oleuropein is one of main polyphenolic antioxidants and the most active phenolic compound of olive leaves.

Chitosan, a compound obtained by various methods, is preferred in the food industry due to its antioxidant and antimicrobial properties. Research on the use of chitosan, an edible film, to increase the shelf life of foods is increasing day by day (Ramezanzade et al., 2021; Silva et al., 2021). Chitosan films can control oxygen and moisture permeability, and these films have been found to have antioxidant and antimicrobial effects on food (Silva et al., 2021).

In this study, the oleuropein was encapsulated into chitosan polymer. The encapsulated chitosan compounds were analyzed by Fourier transform infrared spectroscopy (FTIR), and their molecular weights were determined by the cryoscopic method. The antioxidant activity (%) was determined using the DPPH/trolox. The statistical analysis was applied to the obtained results.

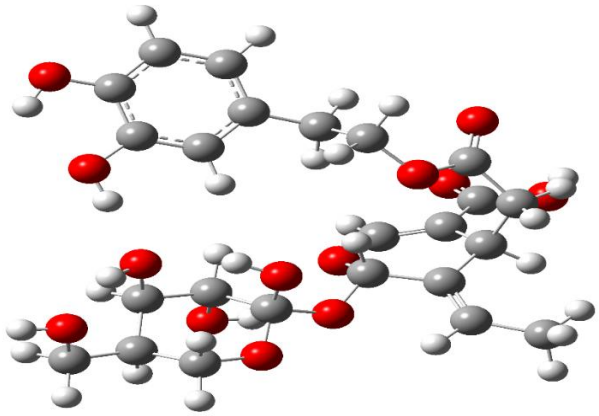

Figure 1. The molecular structure of oleuropein

II. MATERİALS AND METHODS

2.1. Materials

The sodium carbonate, DPPH (1,1-diphenyl-2-picrylhydrazyl), methanol, ethanol, trolox, Hydrochloric acid, sodium tripolyphosphate, hydrochloric acid, sodium hydroxide, acetic acid and chitosan were purchased from Sigma-Aldrich. The oleuropein was extracted from olive leaf.

2. 2. Methods

2.2.1. Preparation of oleuropein extract

The olive leaf sample was first pulverized. The resulting powder sample (10 g) was mixed with 100 mL of methanol. The mixture was homogenized by stirring for 24 hours on a magnetic stirrer and the solvent was removed from the stock solution with the help of an evaporator (Baysal, 2020).

2.2.2. Preparation of oleuropein loaded chitosan nanoparticles

5 gr of chitosan was weighed and after making up with 200 ml of distilled water and adding 25 ml of acetic acid, it was mixed in a magnetic stirrer for 30 minutes at room temperature. Then, while the mixing was continuing, 2 g of oleuropein extract was weighed and the pasteur pipette was added dropwise and mixing was continued for 15 minutes. After 15 minutes, chitosan/TPP 3/1 ratio sodium tripoly phosphate compound (0.5%) was added dropwise while mixing with a pasteur pipette, and the pH value was adjusted to 4.0 with 0.1 N HCl solution and mixing was carried out for 24 hours. After stirring for 24 hours, the cooling program was 18 hours at 0 ^{o}C and 24 hours at −35 ◦C, followed by 24 hours at -80 ◦C. Finally, the chitosan solution was completely lyophilized by freeze drying at -80 ◦C for 72 hours (Baysal et al., 2020). The final product was named CSx.

2.2.3. Characterization

FTIR spectra (Mattson 1000 infrared spectrophotometer) were measured in the wavenumber range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. PG Instruments T60 was used as UV spectrophotometer. LABCONCO Freezone 4.5 is used as freeze dryer.

2.2.4. Determination of antioxidant capacity of oleuropein and chitosan:

60 µM DPPH and 10 µM Trolox solution were prepared using methanol solvent. Control solution was prepared with 3.9 mL of DPPH and 0.1 mL of methanolTo plot the calibration curve, the stock Trolox solution was diluted at concentrations of 0.2 µM, 0.25 µM, 0.4 µM, 0.5 µM, and 0.7 µM. First, 100 µL of solution from each of a total of six test tubes was transferred to six separate test tubes with Trolox solutions, including stock Trolox solution. 3.9 mL of stock DPPH solution was added to each of the tubes and a homogeneous mixture was obtained using vortex. The control solution and these preparations were kept in the dark for 60 minutes. Absorbance values were read at 517 nm in a UV spectrophotometer and a calibration curve was drawn. 1.5 mL and 3.9 mL of stock DPPH solution of extract were added to the test tubes. Prepared mixtures were kept in the dark for 2 hours and absorbance values were read in UV spectrophotometer at 517 nm (Czerwinska et al., 2012).

2.2.5. Determination of Molecular Weights

Cryoscopy (freezing point depression) method was used to determine the molecular weight of the synthesized encapsulated polymers. Pure chitosan and synthesized CSx compounds were weighed 0.5 g and mixed in 25 ml solvent for 1 hour and their freezing points were determined by freezing. The molecular weights were calculated according to the dalton unit with the help of the equation numbered 2 below. (Td= freezing point depression, Kd=molal freezing point falling constant, m=molality)

$$\Delta Td = - Kd \times m_{solute} \quad (2)$$

2.2.6. The Statistical analysis

Minitab 16 software was used for statistical analysis in the research. A one-way ANOVA and Tukey test at 95% confidence level were used to determine the significant difference between the mean values (Peighambardoust et al., 2019).

III. RESULTS AND DİSCUSSİON

3.1. FTIR analysis

Figure 2 shows the FTIR spectra of CSx and chitosan (CS). The sharp peak seen at 3300-3500 cm^{-1} in the spectra appears as the characteristic peak of chitosan and corresponds to the OH bands. Amide II carbonyl bonds observed around 1650 cm^{-1} of chitosan are seen as a small stretch in the spectrum of CSx. The absorption band (amide I) seen at 1650 and 1420 cm^{-1} is due to stretching of carbonyl bonds of acetamide groups and deformation of C-H bonds. The presence of oleuropein used for the encapsulation of chitosan is observed at 2800-2900 cm^{-1} as a double peak with aliphatic CH_2 stretch bonds (Peighambardoust et al., 2019; Pulicharla et al., 2016).

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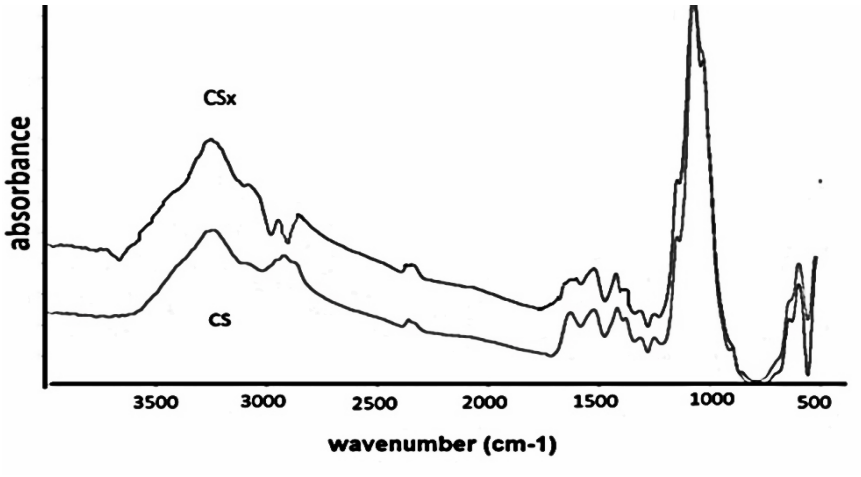

Figure 2. FTIR spectra of chitosan and CSx.

3.2. The determination of % Antioxidant Activity

In antioxidant analysis, trolox antioxidant material was used as a reference solution and a calibration curve was obtained by preparing 6 serial solutions. The R^2 value for trolox was calculated as 0.9914 from the calibration equation. The calibration equation is y = 0.3144x + 0.2743. Table 1 and Figure 2 show the percent antioxidant activity of chitosan samples encapsulated with oleuropein [7, 8].

Table 1. % Antioxidant activity of compounds

(517 nm)

	60 µM	30 µM	15 µM	7.5 µM	3.75 µM
CS	44.02±0.02	52.11±0.002	61.28±0.001	62.22±0.01	62.40±0.001
CSx	55.46±0.001	58.77±0.002	65.24±0.002	65.78±0.001	66.22±0.001
Oleuropein	18.46±0.002	21.4±0.001	22.2±0.001	22.15±0.001	22.04±0.002

* Data are mean of triplicate measurements ± SD. Different alphabetical letters in the columns indicate significant ($p < 0.05$) differences between means in Tukey's test.

As seen in Table 1 and Figure 3, the % antioxidant activity values of the compounds show higher activity as the concentration of the DPPH solution decreases. When the data in the table are compared, the % antioxidant activity of chitosan was 44.02% in 60µM dpph solution, while it reached 62.40% in 3.75 µM dpph solution. A similar increase occurred for oleuropein and CSx compounds.

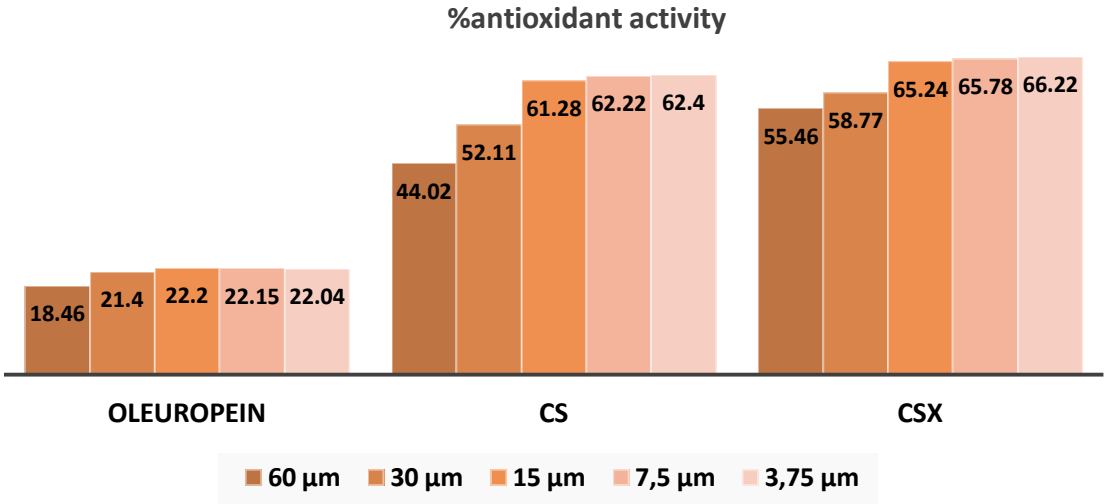

Figure 3. % Antioxidant activities of oleuropein, chitosan (CS) and CSx compounds

3.3. Determination of Molecular Weight

The cryoscopy method called freezing point depression was used as the molecular weight calculation method. The raw and synthesized samples were weighed 0.5 g and the medium was brought to acidic pH with the help of hydrochloric acid in the solvent and mixed for 3 hour. Then, the freezing point was measured in the cooling cabinet with the help of a thermometer. According to the results obtained, the molecular weight of crude chitosan was calculated as 67 kDa. The molecular weights of synthesized CSx was calculated as 88 kDa. Figure 4 shows molecular weight of chitosan (CS) and CSx compounds

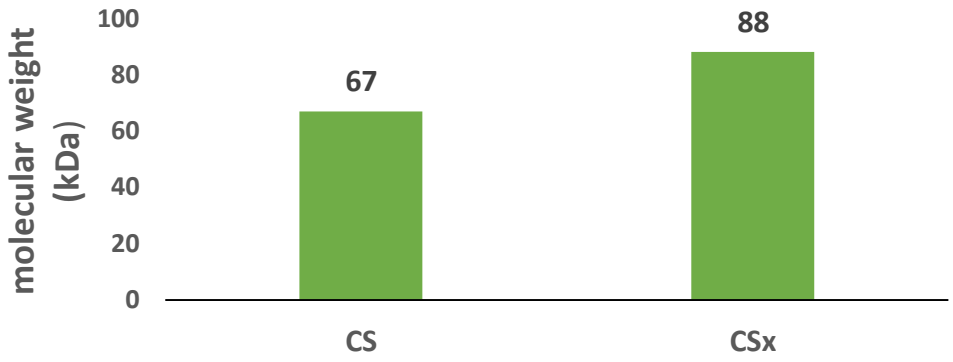

Figure 4. Molecular weight of chitosan (CS) and CSx compounds

IV. CONCLUSİON

According to the analysis results, the % antioxidant activity values of CSx was determined as 66.22%. When the antioxidant activities of CSx and oleuropein compounds were compared, it was determined that oleuropein extract amount and % antioxidant activity were higher in CSx compound. Because the oleuropein and and chitosan have high antioxidant effects. The molecular weights of CSx was calculated as 88 kDa. In this study, it is suggested that the formed compound can be used as packaging material because of antioxidant effect.

CONFLICT OF INTEREST

There is no conflict to disclose.

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