EFFECT OF UV IRRADIATION ON STORAGE OF RIVIERA OLIVE OIL IN GLASS BOTTLES COATED WITH BNNSS/PVB MULTILAYER THIN FILM

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Abstract
UV absorbers are used to reduce the harmful effects of UV rays and to ensure that the properties of the materials are adequately protected. To protect against UV rays, one of the UV absorber molecules can reduce the combined energy of radiation to thermal energy that provides less energy in a photophysical process. Inorganic materials such as metal oxide semiconductors can effectively absorb UV irradiation and exhibit good heat-resistance properties. TiO_2, CeO_2, and ZnO are inorganic compounds with UV protective properties. Since some of these compounds are colored, their use is limited. Boron nitride nanosheets (BNNSs) are alternative UV inhibitors to these inorganic compounds. In this study (BNNSs)/PVB multilayer thin films were produced for the protection of glass packages using the dip-coating method. Riviera olive oil (RO-oil) samples in the both coated and uncoated bottle were exposed to UV irradiation for different times (75, 170, 360 and 720 hours). After the UV irradiation at different times, the total phenol and antioxidant contents in RO-oil were determined by Folin Ciacalteu and DPPH methods. The color change of RO-oil after UV irradiation was determined by UV visible spectrophotometer. Specific absorption coefficients K232 and K270 (specific extinction) in the ultraviolet region were measured for estimating the oxidation stage of RO-oil after degradation. Free fatty acid (FFA) of RO-oil after UV irradiation was determined by the titration method. According to the results of the analysis, the acidity values of RO-oil were preserved 20 times compared to the uncoated glass bottle after 720 hours UV. BNNSs/PVB coating was also effective in preserving total phenol, antioxidant contents and oxidation state of RO-oils under long term UV irradiation.

Keywords: UV irradiation, BNNSs, protective coating, antioxidant properties, riviera olive oil

1. Introduction
Ultraviolet (UV) irradiation as part of sunlight can significantly speed up the aging of many materials and can cause unrecoverable or unhealthy effects of human beings. Recently, the destruction of the ozone layer in the atmosphere of the world has created more risks for the environment, especially in a plant, human and animal life [1]. Since UV radiation triggers the formation of free radicals, prolonged exposure, human skin, food products, etc. may cause serious damage to materials [2]. For this reason, many products that remain in UV radiation for a long time should be protected. Boron nitride nanosheet (BNNSs) is a preferred material for ultraviolet (UV) protection. It can absorb the harmful UV part of sunlight and prevent the surface covered by BNNSs from environmental degradation [3]. There are several studies about the storage of extra virgin olive oils under different conditions and their behavior under UV radiation. In the study of the Vacca et al. effect of extra-pure olive oil on the quality of storage time and the environmental conditions were investigated. Extra pure olive oil was stored in hermetically sealed 60 mL colorless glass bottles. The vials were filled with 3 mL of space and left at direct sunlight or in the dark at 22 ± 3 for 18 months at room temperature. The analyses were carried out after 2, 4, 6, 8, 12 and 18 months of storage as soon as the olive oil samples reached the laboratory. The results of the analysis of fats showed that all parameters underwent significant changes during storage: free acidity, peroxide, and ultraviolet (UV) spectrophotometric indices remained below the limits outlined in the EEC Regulation 2568/91 and 1989/03. Regarding exposure conditions, storage in the dark showed better results in maintaining the quality of oil as expected [4]. Luna et al. researched the effects of UV radiation on the chemical and sensory properties of pure olive oils (Arbequina and Picual). Even small doses of UV radiation have caused oxidation of extra virgin olive oil samples. After the UV degradation, the intensity of pain and fruity sensory properties decreased, and the total phenol and fatty acid contents decreased, whereas the sensory properties of the degraded contents increased [5]. Caponio et al. studied the effect of UV exposure to natural extra virgin olive oil during storage. An experimental study was conducted to examine the effect of light exposure on the quality of the infiltrating oil during a 12-month storage period compared to the quality of the infiltration oil stored in the dark. The results showed that the oils stored under the light had lower tocopherol, carotenoid, and chlorophyll content than those stored in the dark. Furthermore, while the oils stored in the dark contain mainly primary oxidation products, the light-contained oils contain K270 values and secondary oxidation products which exceed the legal limits even after purification with alumina. Overall results show that the shelf life of the oils stored under light is less than that stored in the dark and that the oils can no longer be regarded as leaking after only 2 months of exposure to light [6].
In this paper according to our knowledge from literature long-term UV protective properties of BNNSs/PVB thin films for riviera olive oil storage coated on glass bottles were investigated for the first time. On the other hand, the antioxidant properties of riviera olive oil both storage in coated and uncoated glass bottles under different UV radiation times were determined. BNNSs/PVB coatings showed UV protective properties for RO-oil storage.

2. Materials and Method

2.1. Materials

To prepare immersion solution in the experiments, boron nitride (2,27 g / cm^3,> 98% purity, micron size, Bortek Bor Technologies and Mechatronics Industry Inc.) and isopropyl alcohol (JT Baker) as a solvent were used as the starting material of the nanosheets. Polyvinyl butyral (PVB, ABCR in 0.1% w/w isopropyl alcohol solution) was used to enhance and facilitate the adhesion of boron nitride to the glass surface. Ethanol (99% pure, Sigma Aldrich) and deionized water were used to clean the surface and increase the wettability of the glass. Glass test tubes were used as a glass bottle for storage of olive oil. Riviera olive oil was used from Tariş brand. Folin-Ciocalteu (Merck, 1.24 g / cm^3) reagent and sodium bicarbonate (Na_2CO_3, Merck,> 99%) were used for total phenol analysis. Anhydrous gallic acid (Merck) was used to generate the calibration curve. Antioxidant removal activities were determined by using 1,1-diphenyl-2-picrilhydrazil radical (DPPH) (Sigma Aldrich). DPPH solution was prepared with methanol (J.T. Baker> 99.9%). Tween-20 (Sigma Aldrich, 1.095 g / mL) was added to the extract solution to prevent the oil from crystallizing and to remain stable. Cyclohexane (Fluka Analytical, 99.5%) was used to determine the specific absorbance values of olive oils. Potassium hydrogen phthalate (Sigma Aldrich, 99.95%), potassium hydroxide (Merck,> 85%), diethyl ether (Merck), ethanol (Merck) and phenolphthalein (VWR Prolabo) was used in the determination of free fatty acids in olive oil.

2.2. Preparation of BNNSs/PVB multilayer thin films

Isopropyl alcohol was used as the solvent for the synthesis of BNNSs. 0.2 g of boron nitride was dispersed in 100 mL of solvent (2 mg/mL). The resulting mixture was sonicated for a total of 6 hours at 45 minutes intervals in a 180 W ultrasonic bath (ISOLAB) and centrifuged (Centurion, Series C2) for 30 minutes at 6000 rpm to remove undesirable thickness of boron nitride nanosheets. The supernatant was placed in a new flask. This suspension was used as the dip suspension to form boron nitride nanosheet thin films. Glass bottles to be coated in the thin film formation were washed with detergent water first. The glass surfaces were washed with distilled water and washed with ethanol in ultrasonic bath for 15 minutes to prevent impurities on the glass surface. Completely dry and clean surfaces were immersed in PVB solution for 3 times with 3 mm/s using a dip coating unit (ETOKS Electronics and Software Inc.) to increase adhesion between the material to be coated and the glass surface. Then, at the same rate, glass bottles were immersed in a prepared BNNSs solution for 50 times. The glass bottles were heated in an oven (Protherm) for 2 hours at 400°C.

2.3. UV-A radiation of RO-oil samples

40 mL of olive oil sample was filled into the bottles which were uncoated and coated with BNNSs/PVB and refined under UV light (Philips 36 W PLL-UV A, 315-380 nm) for 75,170,360 and 720 hours. Table 1 contains UV light exposure conditions of olive oil samples.

Table 1. UV light exposure conditions of RO-oil samples

Sample Codes	UV Irradiation Time (hours)	BNNSs coating	Dipping Number
OLR	0	x	-
COLR50-75	75	√	50
UCOLR-75	75	x	-
COLR50-170	170	√	50
UCOLR-170	170	x	-
COLR50-360	360	√	50
UCOLR-360	360	x	-
COLR50-720	720	√	50
UCOLR-720	720	x	-

2.4. Characterization

Total phenol content (TPC) of phenolic extracts of RO-oil was determined by the Folin-Ciocalteau spectrophotometric method in terms of mg GAE / kg oil at 765 nm [7]. 5 g oil sample was weighed for olive oil extraction. 5 mL of methanol /water solution (80:20 v/v) and 3 drops of Tween-20 were added to the oil sample. The sample was sonicated in the ultrasonic bath for 15 minutes and then centrifuged at 5000 rpm for 10 mins. After centrifugation, the supernatant of the sample was collected in a tube. Extraction with residual oil residue was repeated without the addition of Tween 20 and the upper portion of the sample was collected in the same tube containing the previously extracted supernatant. Total phenol content was determined as gallic acid in mg gallic acid/kg oil. Antioxidant removal activity of methanolic extracts with olive oil was evaluated by using Keceli and Gordon (2002) method [8]. 0.1 mL of methanolic extract was added to 2.9 mL of DPPH solution ($6x10^{-5}$ M, methanolic). After keeping in the dark for 30 minutes, the absorbance value was recorded at 515 nm. The color change of RO-oil after UV irradiation was determined by UV visible spectrophotometer (Agilent Technologies, Cary 60 Uv-Vis). The free acidity is expressed as the percentage of total unbound fatty acids in the oils, either as an acid percentage or as an acid number (1 g of KOH required for neutralization of the oil). The free fatty acid analysis was determined in terms of % oleic acid using European Official Methods of Analysis (EEC 1991) [9]. The acidity of the oils was calculated as oleic acid % using the equation given below:

$$oleic\ acid\ \% = \frac{V * N * M}{10 * P}$$

N: concentration of the titrated solution of KOH (mol/L)
M: the molar weight of the oleic acid (282 g/mole)
P: weight of the sample (g)
V : the volume of titrated KOH (mL)

The European Official Analysis Method (Commission Regulation EEC N-2568/91, 1991) was used to determine the specific absorbance coefficients of olive oil samples. 250 mg of olive oil was weighed. The weighed sample was placed in a 25 mL flask and diluted to 25 mL with spectrophotometric grade cyclohexane. The sample was homogenized using vortex for 30 seconds and then the resulting solution was placed in a quartz cuvette. Absorbance at 232 and 270 nm was determined on a UV visible region spectrophotometer using pure cyclohexane as the blank sample. The analyses were carried out in 3 parallel and standard deviation values were calculated.

3. Results and Discussion

3.1. Color change of RO-oil samples

Figure 1. Color change of RO-oil samples

Figure 1 contains color images of riviera olive oil in glass bottles with and without BNNSs/PVB coating 50 times at different UV irradiation times. Visually, it is observed that as the UV irradiation time increases, the colors go from yellow to transparent tone. This was confirmed using UV visible spectroscopy. Transmittance values of each sample at a specific wavelength range were plotted. The absorbance is expected to be high in contrast to low permeability in dark colored samples. The highest permeability values of UCOLR-720 were observed in Figure 2. Transmittance was also higher in the lightest sample as expected. The lowest permeability was observed in the OLR sample before UV degradation.

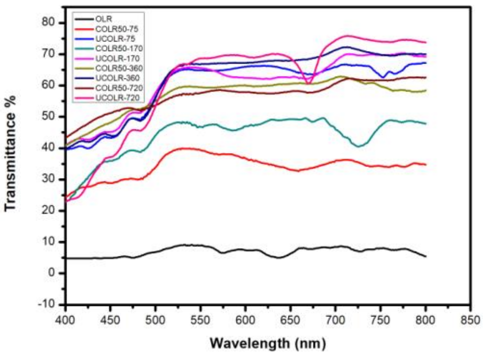

Figure 2. Optical transmittance of riviera olive oil in glass bottles with and without BNNSs/PVB coating at different UV degradation times

3.2. Total phenol content of olive oil samples

Figure 3 contains the total phenol contents of riviera olive oil samples in coated and uncoated glass bottles at different UV irradiation times. When the figure was examined, the phenol content of the olive oil in the uncoated glass showed a significant decrease after 75 hours of UV degradation. The phenol content of riviera olive oil in glass coated with BNNSs/PVB did not change significantly with UV degradation time. After 720 hours of UV degradation of the UCOLR sample, the phenol content decreased by 45.28% compared to the initial state, while the reduction rate for the COLR50 sample was 8.49%.

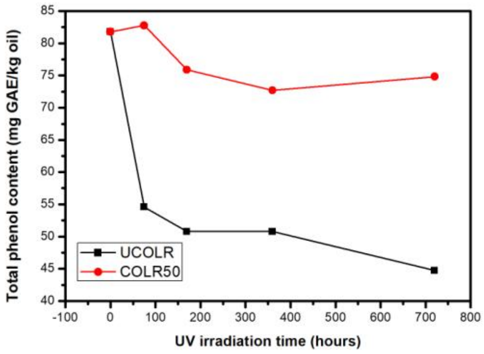

Figure 3. Comparative total phenol content of riviera olive oil in glass bottles with and without BNNSs/PVB coating at different UV irradiation times

These values indicate that the BNNSs/PVB coating is highly effective in preserving phenol contents of riviera olive oils after a UV degradation of 720 hours.

3.3. Antioxidant removal activities of olive oil samples

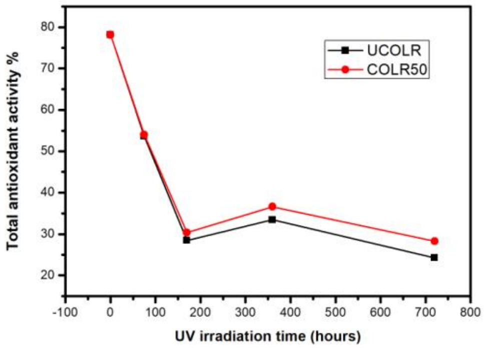

Figure 4. Comparative total antioxidant activity of riviera olive oil in glass bottles with and without BNNSs/PVB coating at different UV irradiation times

Figure 4 shows the total antioxidant activity values of riviera olive oil samples in coated and uncoated glass bottles by dipping 50 times at different UV irradiation times. The highest loss of antioxidant values was observed after the first 75 hours of UV degradation. The antioxidant content of both samples decreased by 30.9% compared to pre-UV degradation. This reduction value after 720 hours of UV degradation was 63.79% for COLR50 and 68.96% for UCOLR. In olive oil, the antioxidant content of olive oil sample in the coated glass bottle was higher after 720 hours than in uncoated glass bottle. Antioxidant activity decreased in both samples after 75 hours, whereas after 170 hours of UV degradation this value was almost fixed and did not change for both samples. BNNSs/PVB coating was effective in preserving total phenol and antioxidant contents under long term UV degradation in riviera samples. In their study, Dabbou et al. confirmed that total phenol content and antioxidant activity decreased in the first months of storage [10].

3.4. Specific absorbance (K232 and K270) values of olive oil samples

To estimate the oxidation stage of olive oil, specific absorption coefficients (specific extinction) in the ultraviolet region need to be determined. The wavelengths of 232 and 270 nm in the ultraviolet region are related to the formation of conjugated diene and triene in the olive oil system due to absorption, oxidation or refining processes. Oxidation compounds of conjugated dienes contribute to K232, while secondary oxidation compounds (aldehydes, ketones, etc.) contribute to K270 [11].

Figure 5 shows the K232 values of riviera olive oil samples in coated and uncoated glass bottles at different UV irradiation times. When K232 values for riviera oil were examined, K232 values increased for 170 hours after UV degradation for both samples. The K232 value of the COLR50 sample tended to stabilize after 360 hours, while the K232 value of the UCOLR sample continued to increase.

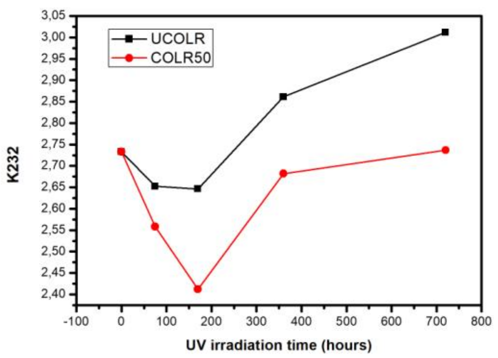

Figure 5. Comparative K232 values of riviera olive oil in coated and uncoated glass bottles at different UV irradiation times

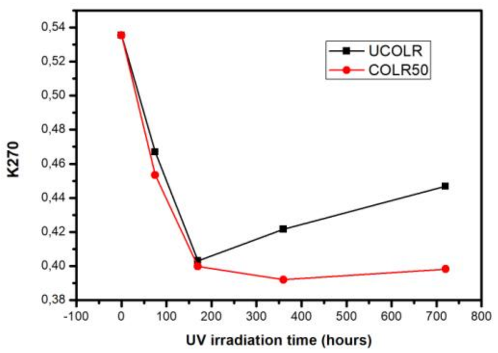

Figure 6. Comparative K270 values of riviera olive oil in coated and uncoated glass bottles at different UV irradiation times

When the K270 values are compared in Figure 6, it can be said that it is possible to see the effect of the coating after 170 hours. After 170 hours of UV degradation, the K270 values of the UCOLR sample, the number of secondary oxidation products, increased, while the constant pattern increase was not observed in the COLR50 sample. After 720 hours of UV degradation, the K270 value of the UCOLR sample was 0.4468, while the COLR50 sample remained at 0.3982. Although K232 and K270 values are based on the determination of diene and triene conjugated compounds, it is very difficult to evaluate the analysis results based on these results only in riviera and refined oils because these products can form during deodorization and bleaching [12].

3.5. Free fatty acid content of olive oil samples

The amount of free fatty acid (FFA) is one of the most important factors to determine the quality of olive oil.

According to the literature, free fatty acidity is a measure of oil quality and reflects the oil production and storage processes, oil balance and sensitivity to deterioration. Fatty acids are long-chain structures consisting of carbon atoms. The amount of free fatty acids increased as the UV irradiation time increased. This increase in acidity is due to the hydrolysis of free fatty acid. It helps to separate fatty acids from triglyceride molecule by the effect of natural enzymes in olive fruit. The effect of lipase is that it produces free fatty acids which are responsible for the acidity of the oil [13].

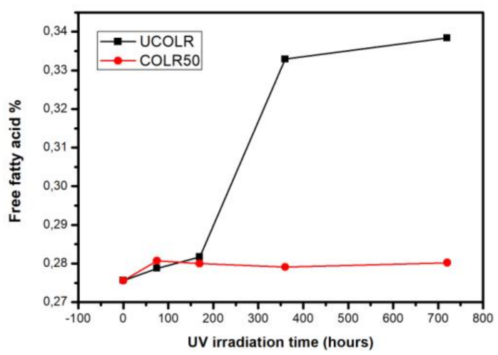

Figure 7. Comparison of free fatty acid values of riviera olive oils in glass bottles with and without BNNSs/PVB coating at different UV irradiation times

Figure 7 shows the free fatty acid values of riviera olive oil in glass bottles with and without BNNSs/PVB coating at different UV irradiation times. While the amount of free fatty acids in the extra virgin olive oils was around 0.6%, this value was maximum 0.33% in riviera oils. Figures 7 interpret in the first 170 hours of UV degradation, the % oleic acid content of the UCOLR and COLR50 samples was almost the same and there was not much increase compared to the pre-UV degradation. After 170 hours the acidity values of the COLR50 sample remained constant, while the acidity value of the riviera olive oil in the uncoated glass was increased by up to 360 hours. After 720 hours UV, the free fatty acid value of the UCOLR sample increased by 22.78% compared to the initial state, while the increase in the COLR50 sample was only 1.67%.

Due to the high oxygen permeability of olive oils in uncoated glass, they cause oxidation of triglycerides [14]. High oxygen concentration accelerates the formation and degradation of hydroperoxides, providing the formation of carboxylic acids that influence the increase of acidity [15].

4. Conclusions

- The highest transmittance values were observed in UCOLR-720 sample. Transmittance was also higher in the lightest sample as expected. The lowest transmittance was observed in the OLR sample before UV degradation.
- After 720 hours of UV degradation of the UCOLR sample, the phenol content decreased by 45.28% compared to the initial state, while the reduction rate for the COLR50 sample was 8.49%. These values show that after 30 days of UV degradation, BNNSs/PVB coating is very effective in preserving phenol contents of riviera olive oils.
- BNNSs/PVB coating was effective in preserving total phenol and antioxidant contents under long term UV degradation in riviera samples.
- The K232 value of the COLR50 sample tended to stabilize after 360 hours while the K232 value of the UCOLR sample continued to increase. After 170 hours of UV degradation, the K270 values of the UCOLR sample as well as the number of secondary oxidation products, increased, while the constant pattern increase was not observed in the COLR50 sample.
- When the acidity amounts of riviera olive oil were compared, the free fatty acid value of UCOLR sample increased by 22.78% after 720 hours UV and the increase in COLR50 sample was 1.67%. BNNSs/PVB coating in riviera olive oil protected the free acidity value against long-term UV degradation.

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