

Oxidative stability of UV irradiated and X-rayed soybean oil incorporated with rose oil

M.T. Golmakani^{a,✉}, S. Barani^a, N. Alavi^a and Z. Tahsiri^a

^aDepartment of Food Science and Technology, School of Agriculture, Shiraz University, Shiraz, Iran.

✉Corresponding author: golmakani@shirazu.ac.ir

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SUMMARY: The effects of UV radiation and X-ray on the oxidative stability of soybean oil were investigated. Also, rose oil was incorporated into soybean oil and its antioxidant activity was compared with that of α -tocopherol during accelerated storage. Treating the samples with radiation (UV and X-ray) stimulated the oxidation process in soybean oil in comparison with samples that did not receive radiation. X-rayed samples had significantly higher amounts of oxidation products than UV irradiated samples. The X-ray caused more oxidation in the samples due to its higher energy content. Also, the antioxidant activity of rose oil was comparable with that of α -tocopherol.

KEYWORDS: Oxidation; Radiation; Rose oil; Soybean oil; UV; X-ray

RESUMEN: *Estabilidad oxidativa del aceite de soja conteniendo aceite de rosa e irradiado con UV y rayos X.* Se investigaron los efectos de la radiación UV y los rayos X sobre la estabilidad oxidativa del aceite de soja. Además, el aceite de rosa se incorporó al aceite de soja y su actividad antioxidante se comparó con la del α -tocoferol durante el almacenamiento acelerado. El tratamiento de las muestras con radiación (UV y rayos X) fomentó el proceso de oxidación del aceite de soja en comparación con las muestras que no recibieron radiación. Las muestras con rayos X tenían cantidades significativamente más altas de productos de oxidación que las muestras irradiadas con UV. Los rayos X causaron más oxidación en las muestras debido a su mayor contenido de energía. Además, la actividad antioxidante del aceite de rosa fue comparable con la del α -tocoferol.

PALABRAS CLAVE: Aceite de rosa; Aceite de soja; Oxidación; Radiación; Rayos-X; UV

ORCID ID: Golmakani MT <https://orcid.org/0000-0001-5173-1178>, Barani S <https://orcid.org/0000-0001-5349-2182>, Alavi N <https://orcid.org/0000-0002-8786-5028>, Tahsiri Z <https://orcid.org/0000-0002-5534-8448>

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1. INTRODUCTION

Irradiation involves the process of exposing raw and processed foods to ionizing and non-ionizing radiation. X-rays, gamma rays and electron beams are ionizing types of radiation, whereas UV, visible

light, infrared, and microwave are non-ionizing types (Alothman *et al.*, 2009). Radiation can affect foods that are susceptible to lipid oxidation. It can also induce excitation and ionization, as well as the production of free radicals (Richards, 2005). Lalas *et al.*, (2007) evaluated the effects of gamma rays

on olive, sunflower and soybean oils (SBO). They reported that gamma rays can shorten the induction period (IP). Hashemi *et al.*, (2011) evaluated UV radiation on rapeseed oil. Their results showed that the oxidation process intensified when the irradiated rapeseed oil was considered.

According to FAOSTAT, the worldwide production of SBO was 45.70 million tons in 2014. SBO is used in various foods including margarine, cooking and salad oils, mayonnaise and salad dressings. SBO is known for its low stability due to its high unsaturation content (Hammond *et al.*, 2005; O'Brien, 2008). Also, the stability of SBO depends on different factors including handling, processing and its composition (Hammond *et al.*, 2005).

Rosa damascena Mill. (Damask rose) is from the Rosaceae family and has been cultivated since ancient times in Iran, India, Turkey and Bulgaria. This fragrant rose is a member of a few rose species produced commercially for oil, hips, concrete, absolute, and rose water (Mahboubi, 2016). The available literature has revealed that rose oil shows antioxidant (Yassa *et al.*, 2009), antibacterial (Ulusoy *et al.*, 2009), antifungal (Mahboubi *et al.*, 2011) and anticancer (Zu *et al.*, 2010) activities. The components of rose oil vary in different parts of the world. In Iran, the main components of rose oil have been reported to be citronellol (14.5-48.2%), nonadecane (10.5-40.5%), geraniol (5.5-18%), and heneicosane (5-14%) (Mahboubi, 2016). In this context, Wei and Shibamoto (2007) investigated the antioxidant capacity of rose oil. Their results showed that citronellol was the main component of rose oil with high antioxidant capacity. In addition, they reported that rose oil could inhibit hexanal oxidation in the aldehyde/carboxylic acid assay. It could also inhibit the formation of malonaldehyde from squalene when treated with UV radiation.

To the best of our knowledge, there are no reports on the effects of UV radiation and X-ray on the oxidative stability of SBO incorporated with rose oil. Given the varied impacts of ionizing and non-ionizing types of radiation on the process of oxidation in oils, the objective of the present study was to evaluate the oxidative stability of SBO by applying UV radiation and X-rays to the oil samples. Also, the antioxidant capacity of rose oil was compared with that of α -tocopherol.

2. MATERIALS AND METHODS

2.1. Materials and methods

All experimental chemicals were of analytical grade and were purchased from Merck Company (Darmstadt, Germany) and Sigma-Aldrich Company (St. Louis, MO). The SBO was refined, bleached and deodorized, while it did not

contain any added antioxidants. The SBO was provided by Narges Shiraz Oil Company and the rose oil was provided by the Manely Company.

2.2. Rose oil

2.2.1. Chemical composition

A gas chromatography (GC) (7890A, Agilent Technologies, Santa Clara, CA) was equipped with a HP-5MS capillary column (5% Phenyl Poly silphenylene-siloxane; 30 m length \times 0.25 mm internal diameter; 0.25 μ m film thickness) and was used accordingly. A mass spectrometer detector (5975C, Agilent Technologies, Santa Clara, CA) was operated at 70 eV electron ionization energy, in the electronic ionization mode, 0.5 s/scan, and a mass range of 50–480 atomic mass units. One μ L of rose oil was injected into the GC/MS. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector was in split mode (at a ratio of 1:50) and its temperature was 280 °C. The oven temperature was programmed to increase from 60 °C to 210 °C at a rate of 3 °C/min. The temperature was finally increased to 240 °C at the rate of 20 °C/min and held at this temperature for 8.5 min. The total running time was 60 min. The interface line temperature was 280 °C. The MSD ChemStation Software (G1701EA, E.02.01.1177, Agilent Technologies, and Santa Clara, CA) was applied to analyze mass spectra and chromatograms. The compounds were identified by comparing their mass spectral fragmentation patterns with those in the data bank (Wiley/NBS). A quantitative analysis of EO compounds was made under the same chromatographic conditions using a GC coupled with a flame ionization detector (FID). The relative data in percentages were compiled from the electronic integration of the chromatogram peak areas.

2.2.2. Radical scavenging activity

DPPH° (2,2-diphenyl-1-picrylhydrazyl radical) scavenging activity of rose oil was evaluated according to the method described by Eblaghi *et al.*, (2016) with some modifications. Accordingly, three mL of methanolic solution consisting of different concentrations of rose oil were mixed with one mL DPPH° solution.

The IC₅₀ value was calculated as a rose oil concentration that could provide 50% inhibition of the DPPH° activity. This is obtained from the graph plotting of the inhibition percentage against the concentration of rose oil.

2.3. Initial chemical properties of SBO

The peroxide value (PV) and the anisidine value (AnV) were calculated according to the AOCS

(American Oil Chemists' Society) Official Methods Cd 8-53 and Cd 18-90, respectively (AOCS, 2000). The Totox value ($TV = 2PV + AnV$) is determined as the total amount of oxidation, including primary and secondary oxidation products (Shahidi and Zhong, 2005). Specific extinction coefficients at 232 nm (K_{232}) and 268 nm (K_{268}) were determined according to the AOCS Official Method Ch 5-91 (AOCS, 2000).

To determine the fatty acid composition, fatty acid methyl esters (FAMES) of SBO were prepared according to the method described by Golmakani *et al.*, (2012). For this purpose, a GC system (SP-3420A, Beijing, China) was equipped with an FID and a BPX-70 fused silica capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness) and was used accordingly. Nitrogen was the carrier gas. The temperatures of injector and detector were 250 and 300 °C, respectively. The injector was in the split mode (a split ratio of 1:10) and 1 μL of FAMES was injected. The oven temperature was programmed as follows: the initial temperature was held at 140 °C for 5 min. Thereafter, the temperature was increased to 180 °C at a rate of 20 °C/min and held for 9 min. Finally, the temperature was increased to 200 °C at a rate of 20 °C/min and held for 3 min. The result was expressed as percentages of the relative peak areas. Each fatty acid was identified based on its retention time in comparison with the standard.

2.4. Accelerated storage of SBO samples

SBO was divided into 3 groups of samples prepared separately: the control, rose oil (1000 mg/kg), and α-tocopherol (100 mg/kg). Each sample was divided into three equal portions, i.e. (a) non-irradiated samples, (b) X-rayed samples (exposed to 1 KGy and 140 kVp, radiography X-ray machine, radiography system, Gilardoni, Italy), and (c) UV irradiated samples (exposed to 31.40 KGy UVC, energy content of 8 eV, TUV30W G30T8, Philips lightening, Amsterdam, Netherland). Samples were then stored in an oven with a temperature that remained at 65 ± 1 °C under dark conditions for 21 days. The indicators of lipid oxidation were namely PV, AnV, and TV. These indicators were measured weekly. The K_{232} and K_{268} of SBO samples were determined at the end of storage.

The effectiveness of all the tested antioxidants was expressed as the F_{value} according to eq. (1):

$$F_{\text{value}} = \frac{IP_s}{IP_c} \quad \text{eq. (1)}$$

where IP_s is the induction period (IP) of the SBO containing antioxidants (rose oil and α-tocopherol) and IP_c is the IP of the control, i.e.

the SBO without any antioxidants. A PV higher than 20 indicates the poor flavor of SBO (O'Brien, 2008). Therefore, IP was calculated as the number of days required for a sample to reach a PV of 20 meq O_2 /kg Oil.

Antioxidant activity (AA) is a function of an antioxidant's concentration and was calculated according to the following equation:

$$AA = \frac{IP_s - IP_c}{[AH]IP_c} \quad \text{eq. (2)}$$

where [AH] is the antioxidant concentration in proper units (Antolovich *et al.*, 2002).

2.5. Statistical analysis

All tests were carried out in triplicate and mean values were calculated. SAS (Statistical Analysis Software, version 9.1; SAS Institute Inc. Cary, NC) was used for statistical analysis and significant differences were calculated using Duncan's multiple range test ($P < 0.05$). Standard deviation values were presented in the tables and the standard deviation bars were provided in the figures.

3. RESULTS AND DISCUSSION

3.1. Rose oil

3.1.1. Chemical composition

Table 1 shows the chemical composition of rose oil. Citronellol (oxygenated monoterpene; 36.68%), n-nonadecane (Alkane; 19.30%), n-heneicosane (Alkane; 9.39%), 1-nonadecene (Alkene; 7.13%), and geraniol (oxygenated monoterpene, 4.48%) were the most abundant chemical components identified in the rose oil. Sadraei *et al.* (2013) identified the chemical components of rose oil and reported that β-citronellol (23%), nonadecane (16%), geraniol (16%) and heneicosane (5%) were its main components.

3.1.2. Radical scavenging activity

The IC_{50} value of rose oil was 4.10 ± 0.70 mg/mL. Accordingly, rose oil can scavenge free radicals. The antioxidant properties of rose oil can be attributed to its chemical components such as citronellol, which present high antioxidant capacity (Wei and Shibamoto, 2007).

3.2. Initial chemical properties of SBO

The PV was 1.99 meq O_2 /kg (Table 2). The GC/FID results indicated that linoleic acid (52.53%) was the major fatty acid in SBO. Considering the

TABLE 1. Chemical composition of rose oil

No.	Chemical compound	Retention index	Relative peak area (%)
1	Citronellol	1226	36.68 ± 4.67*
2	n-Nonadecane	1905	19.30 ± 1.36
3	n-Heneicosane	2103	9.39 ± 0.40
4	1-Nonadecene	1872	7.19 ± 0.81
5	Geraniol	1255	4.52 ± 0.13
6	n-Heptadecane	1700	3.84 ± 0.49
7	n-Eicosane	2005	2.41 ± 0.03
8	Phenyl ethyl alcohol	1108	2.28 ± 0.13
9	Methyl eugenol	1405	2.26 ± 0.19
10	n-Tricosane	2301	1.73 ± 0.24
11	Eugenol	1358	1.54 ± 0.20
12	n-Pentadecane	1497	0.69 ± 0.05
13	Germacrene D	1478	0.60 ± 0.03
14	n-Octadecane	1797	0.49 ± 0.06
15	trans-Rose oxide	1125	0.46 ± 0.01
16	Citronellyl acetate	1353	0.45 ± 0.06
17	n-Pentacosane	2502	0.43 ± 0.03
18	Linalool	1098	0.42 ± 0.02
19	10-Heneicosene	2091	0.37 ± 0.03
20	1-Eicosene	1973	0.36 ± 0.05
21	1-Tricosene	2289	0.31 ± 0.04
22	Geranyl acetate	1382	0.30 ± 0.02
23	n-Docosane	2199	0.27 ± 0.01
24	α-Guaiene	1436	0.26 ± 0.03
25	Limonene	1026	0.26 ± 0.01
26	(E)-Caryophyllene	1416	0.25 ± 0.03
27	(Z,Z)-Farnesol	1719	0.23 ± 0.00
28	Phenyl ethyl octanoate	1847	0.23 ± 0.01
29	α-Humulene	1451	0.22 ± 0.02
30	α-Bulnesene	1503	0.29 ± 0.04
31	n-Hexadecane	1597	0.21 ± 0.03
32	Terpinen-4-ol	1174	0.19 ± 0.01
33	Heptanal	901	0.19 ± 0.01
34	Neryl acetate	1364	0.18 ± 0.02
35	n-Tetracosane	2401	0.15 ± 0.00
36	(E)-7-Octadecene	1772	0.15 ± 0.02
37	Methyl geranate	1323	0.14 ± 0.00
38	α-Pinene	931	0.14 ± 0.01
39	Benzyl benzoate	1760	0.13 ± 0.01
40	α-Terpineol	1188	0.12 ± 0.02
41	β-Elemene	1390	0.10 ± 0.01
42	Nerol oxide	1151	0.10 ± 0.01
43	n-Nonanal	1102	0.10 ± 0.00

*Mean ± standard deviation; Number of replicates: 2; Statistical test: ANOVA and multiple comparison of means using Duncan's test; Degree of significance: $P < 0.05$.

TABLE 2. Initial characteristics of soybean oil

Characteristic	Amount
Peroxide value (meq O ₂ /kg)	1.99±0.22*
Anisidine value (mg/kg)	2.17±0.13
Totox value	6.15±0.17
K ₂₃₂	0.10±0.00
K ₂₆₈	0.16±0.00
Fatty acid composition (%)	
Palmitic acid; C16:0	11.05
Stearic acid, C18:0	4.99
Oleic acid; C18:1 (ω-9)	24.53
Linoleic acid; C18:2 (ω-6)	52.53
α-Linolenic acid; C18:3 (ω-3)	6.43
Σ Saturated fatty acids	16.04
Σ Monounsaturated fatty acids	24.53
Σ Polyunsaturated fatty acids	58.96

*Mean ± standard deviation; Number of replicates for each analysis: 3; Statistical test: ANOVA and multiple comparison of means using Duncan's test; Degree of significance: $P < 0.05$.

unsaturation degree as an indicator, it was observed that polyunsaturated fatty acids (PUFA) comprised most of the fatty acids in SBO.

3.3. Accelerated storage of SBO samples

3.3.1. Peroxide Values

Figure 1 shows the PVs of non-irradiated, UV irradiated and X-rayed SBO samples. X-rayed samples had the highest PVs (Figure 1c), while UV irradiated samples exhibited significantly high PVs (Figure 1b) compared to non-irradiated samples (Figure 1a). Radiation intensified the oxidation process in SBO compared to the condition of non-radiation. Similarly, Zhang *et al.*, (2006) evaluated the effects of UV radiation on the amount of β-sitosterol oxides induced in soybean, sunflower, olive and rapeseed oils. Their results showed that UV radiation can significantly induce the oxidation process.

Except for X-rayed samples, the PVs of all other SBO samples were significantly increased during storage. Although the PVs of X-rayed samples showed an increasing trend until day 14 of storage, they decreased thereafter until the end of storage. The higher energy content of X-ray (Fan, 2012) caused instability and therefore promoted the decomposition of hydro-peroxides into secondary oxidation products (Figure 1c).

Rose oil and α-tocopherol had significantly lower PVs than the control. However, there were no significant differences between the PVs of rose oil and α-tocopherol.

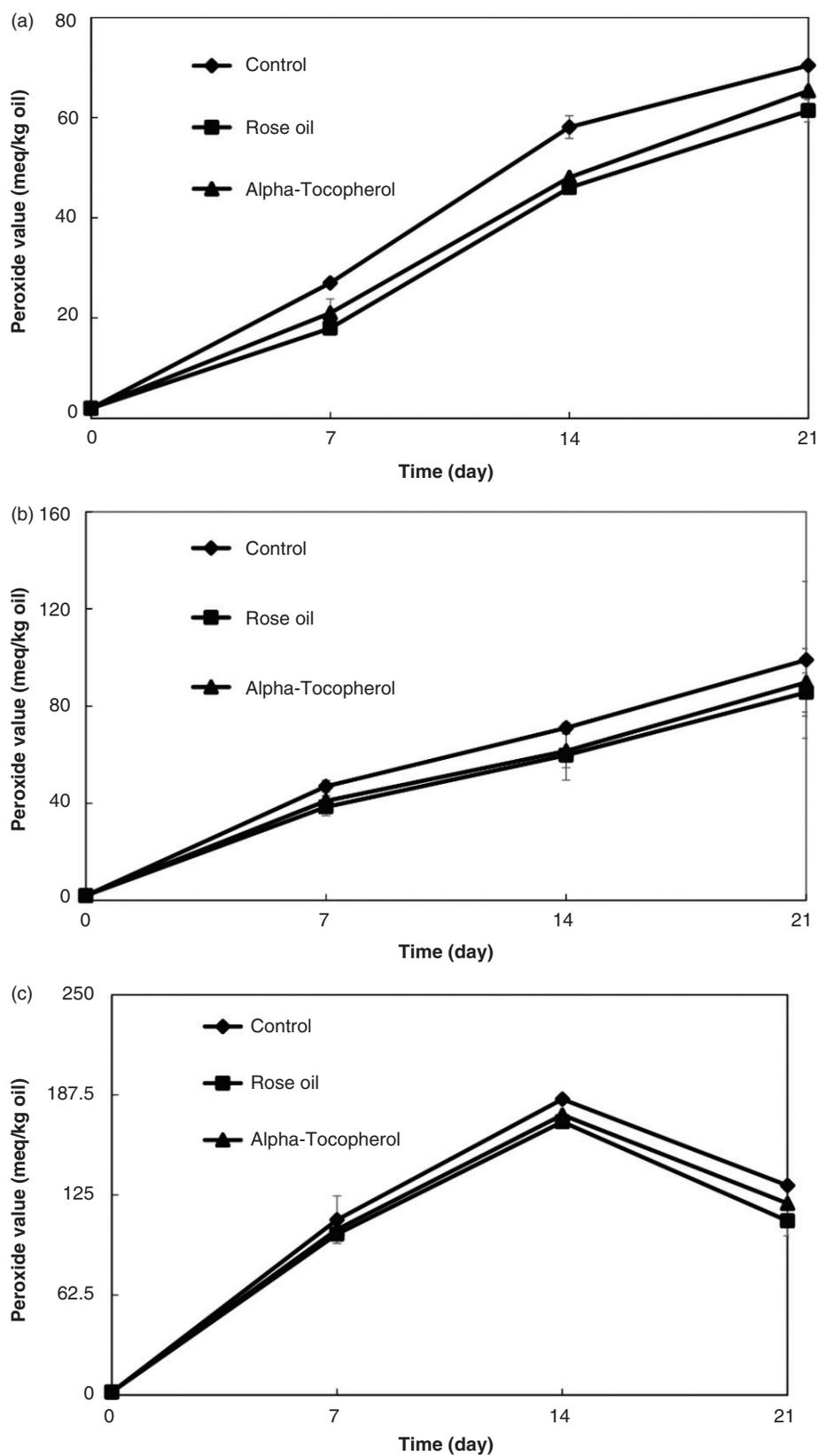


FIGURE 1. Changes in peroxide values of (a) non-irradiated, (b) UV irradiated, and (c) X-rayed soybean oil samples during accelerated storage (Mean \pm standard deviation; Number of replicates for each analysis: 3; Statistical test: ANOVA and multiple comparison of means using Duncan's test; Degree of significance: $P < 0.05$).

3.3.2. Induction period, F_{value} , and antioxidant activity

Table 3 shows the IP, F_{value} , and AA of the SBO samples. Irradiated samples had lower IPs than non-irradiated samples. Accordingly, there is a possible interaction between the rays and SBO which can explain the negative effects of X-ray and UV on SBO stability. Similar to our findings, Gromadzka *et al.*, (2010) studied the effect of UV radiation on the IP of sunflower and rapeseed oils. They found that the IP of UV irradiated samples was lower. According to Table 3, the UV irradiated samples had significantly higher IPs than X-rayed samples. Because of the strong effect of oxidation, rose oil and α -tocopherol were not significantly different.

The F_{values} of irradiated samples were lower than those of non-irradiated samples. The F_{value} of rose oil was significantly higher than that of the control. Therefore, rose oil can be regarded as an effective antioxidant in this context because of its ability to improve the oxidative stability of SBO. In this respect, significant differences were not observed between the F_{values} of rose oil and α -tocopherol.

X-rayed samples had lower AAs compared to non-irradiated samples. Although there were no significant differences between the AAs of rose oil and the control, α -tocopherol caused a significantly higher AA. Since AA depends on the concentration of antioxidants, the AA of α -tocopherol was used at a lower concentration and therefore became significantly higher than rose oil.

3.3.3. Anisidine values

The AnVs of the SBO samples during accelerated storage are illustrated in Figure 2. The X-rayed samples had significantly higher AnVs (Figure 2c) than the UV irradiated samples (Figure 2b).

Non-irradiated samples represented the lowest AnVs (Figure 2a). The higher energy content of X-rays caused the creation of a significantly higher amount of secondary oxidation products. An increase in the AnVs of the X-rayed samples was observed after 14 days. This can be attributed to the decomposition of primary oxidation products into secondary ones (with reference to the negative slope in Figure 1c).

The AnVs of the samples increased significantly with a longer storage time. Although there were no significant differences between the AnVs of rose oil and α -tocopherol, the AnVs of both rose oil and α -tocopherol were significantly lower than that of the control. Keramat *et al.*, (2016) evaluated the effects of *Bunium persicum* and *Rosmarinus officinalis* essential oils on the oxidative stability of virgin olive oil. They reported that the AnVs of essential oils were significantly lower than that of the control.

3.3.4. Totox value

Figure 3 shows the TVs of the SBO samples during accelerated storage. The X-rayed samples had the highest TVs (Figure 3c). The non-irradiated samples had significantly lower TVs (Figure 3a) than the irradiated samples (Figures 3b and 3c). The TVs of the samples were significantly increased by longer durations of storage. The TVs of the SBO samples treated with rose oil and α -tocopherol were similar while being significantly lower than that of the control. As a result, rose oil can be used as a natural antioxidant for reducing primary and secondary oxidation products.

3.3.5. K_{232} and K_{268}

Table 3 shows the K_{232} and K_{268} values of the SBO samples at the end of the storage period. X-rayed

TABLE 3. Effects of UV radiation and X-ray on induction period (IP), F_{value} , and antioxidant activity (AA) of soybean oil

Sample	IP (day)	F_{value}	AA	K_{232}	K_{268}
Non-radiation					
Control	4.76 ± 0.11 ^{b*}	1.00 ± 0.00 ^b	0.00 ± 0.00 ^b	13.91 ± 1.60 ^b	1.76 ± 0.29 ^b
Rose oil	6.48 ± 0.20 ^a	1.36 ± 0.04 ^a	3.62 ± 0.42 ^b	14.61 ± 0.98 ^b	1.54 ± 0.20 ^b
α -Tocopherol	5.96 ± 0.30 ^a	1.26 ± 0.06 ^a	25.11 ± 6.34 ^a	14.40 ± 1.79 ^b	1.45 ± 0.25 ^b
UV radiation					
Control	2.71 ± 0.47 ^d	1.00 ± 0.00 ^d	0.00 ± 0.00 ^b	13.08 ± 0.01 ^b	1.07 ± 0.68 ^b
Rose oil	3.73 ± 0.63 ^c	1.38 ± 0.23 ^c	3.75 ± 2.33 ^b	17.74 ± 0.08 ^b	2.21 ± 0.13 ^b
α -Tocopherol	3.47 ± 0.25 ^c	1.28 ± 0.10 ^c	28.05 ± 9.40 ^a	17.96 ± 0.10 ^b	2.25 ± 0.15 ^b
X-ray					
Control	0.97 ± 0.38 ^e	1.00 ± 0.00 ^f	0.00 ± 0.00 ^d	20.91 ± 2.69 ^a	6.27 ± 0.38 ^a
Rose oil	1.10 ± 0.07 ^e	1.14 ± 0.08 ^e	1.34 ± 0.74 ^d	19.48 ± 0.40 ^a	5.62 ± 0.00 ^a
α -Tocopherol	1.06 ± 0.06 ^e	1.09 ± 0.06 ^e	8.76 ± 6.56 ^c	18.76 ± 0.68 ^a	6.15 ± 0.08 ^a

*Mean ± standard deviation; Number of replicates for each analysis: 3; Statistical test: ANOVA and multiple comparison of means using Duncan's test; In each column, means with different letters are significantly different ($P < 0.05$).

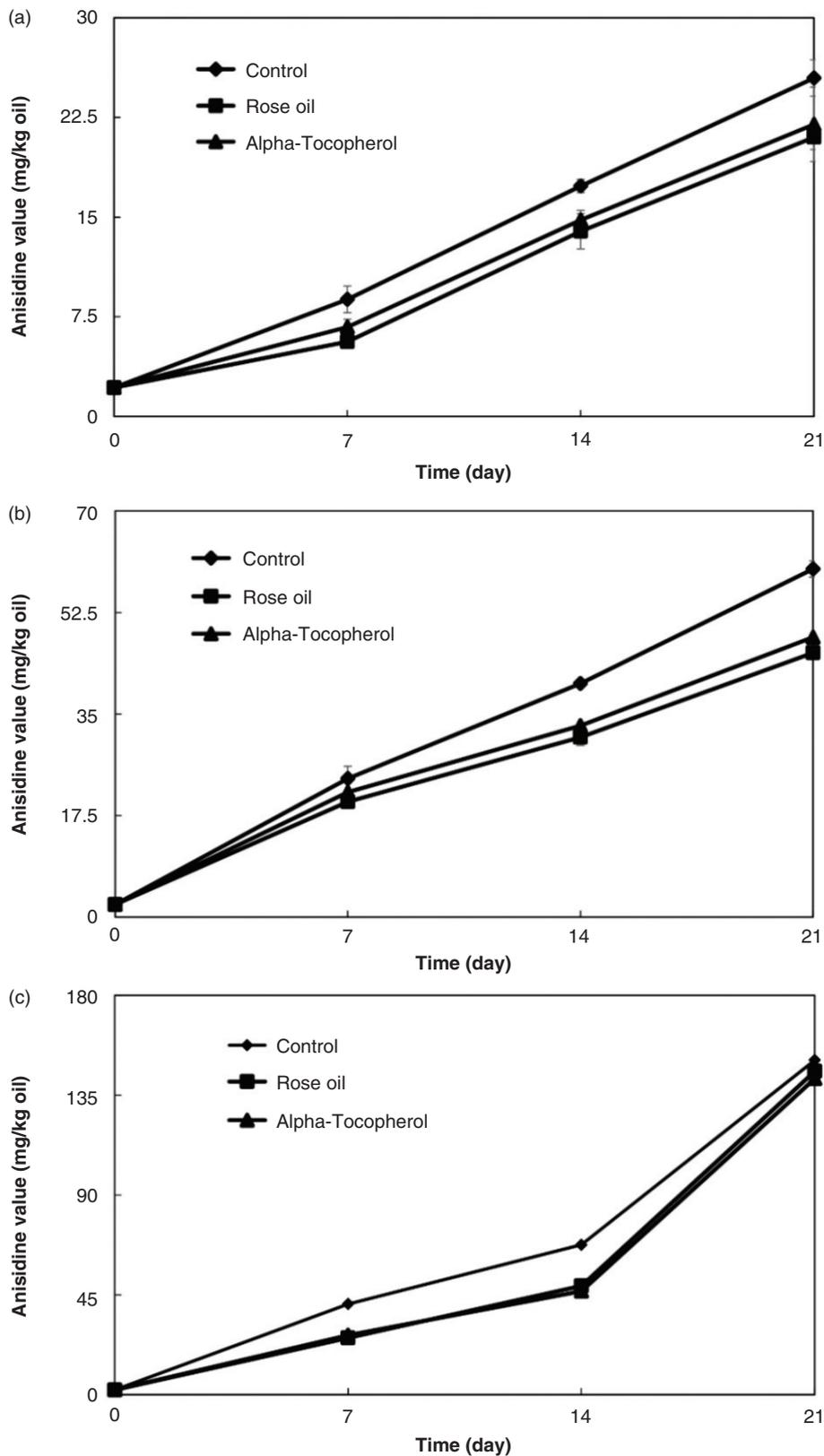


FIGURE 2. Changes in anisidine values of (a) non-irradiated, (b) UV irradiated, and (c) X-rayed soybean oil samples during accelerated storage (Mean \pm standard deviation; Number of replicates for each analysis: 3; Statistical test: ANOVA and multiple comparison of means using Duncan's test; Degree of significance: $P < 0.05$).

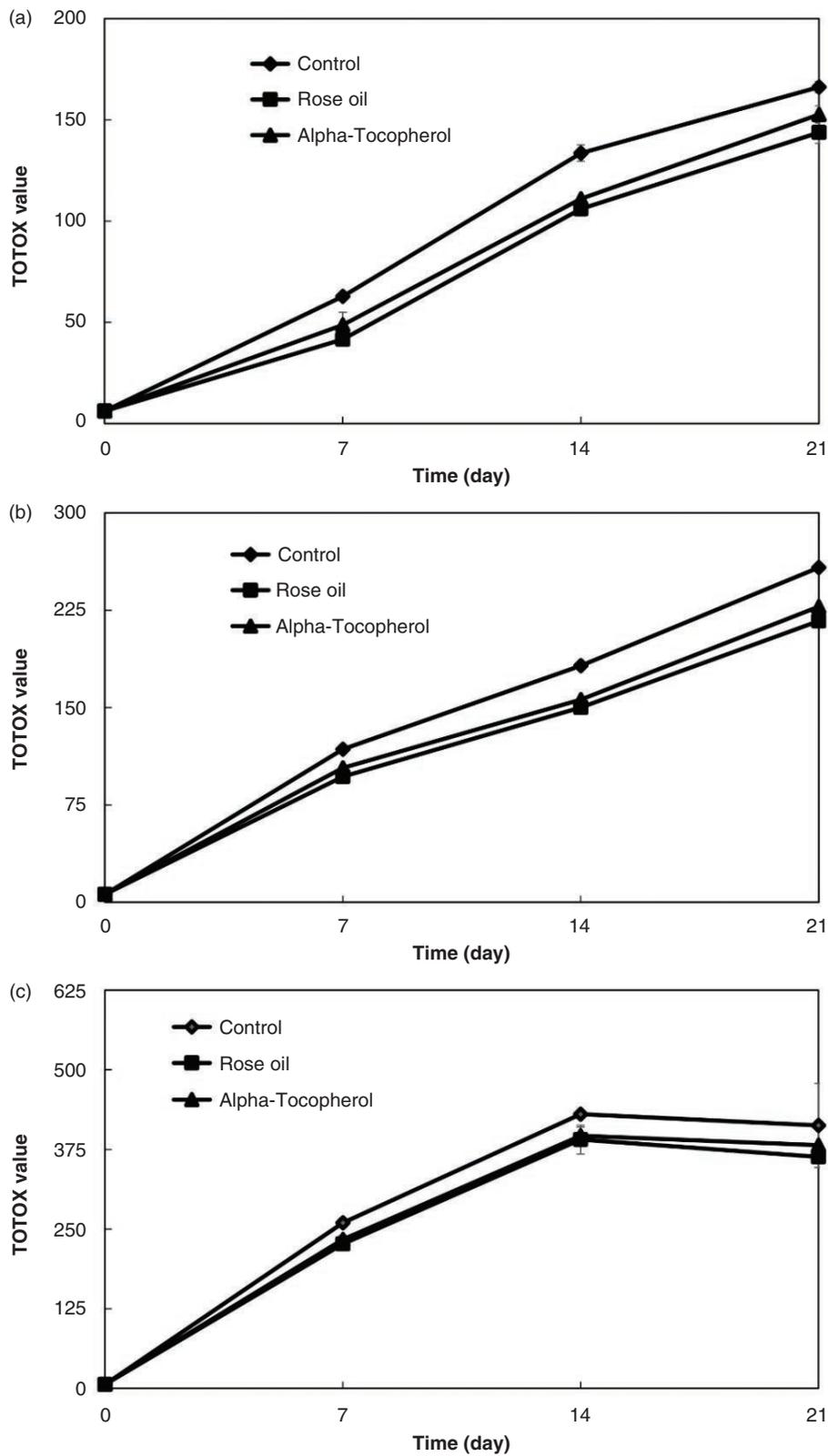


FIGURE 3. Changes in Totox values of (a) non-irradiated, (b) UV irradiated, and (c) X-rayed soybean oil samples during accelerated storage (Mean \pm standard deviation; Number of replicates for each analysis: 3; Statistical test: ANOVA and multiple comparison of means using Duncan's test; Degree of significance: $P < 0.05$).

samples had the highest K_{232} values. In this regard, Braunrath *et al.*, (2010) previously used gamma rays to evaluate the PV, AnV, and conjugated dienes of the triacylglycerols in rapeseed oil. They reported that gamma rays produced primary and secondary oxidation products. In the current study, the K_{232} values of non-irradiated and UV irradiated samples were not significantly different. Furthermore, significant differences were not detected among the K_{232} values of the control compared with those of the samples treated with either rose oil or α -tocopherol.

The K_{268} values of the X-rayed samples were significantly higher than those found in non-irradiated and UV irradiated samples. The K_{268} values of the control, rose oil, and α -tocopherol were not significantly different.

4. CONCLUSIONS

Effects of UV radiation and X-ray were evaluated on the oxidative stability of SBO incorporated with rose oil. Radiation induced higher levels of oxidation processes in the SBO samples. Furthermore, the exposure of samples to X-ray caused significantly higher amounts of oxidation products compared to samples exposed to UV irradiation. The higher intensity of oxidation that occurred in the X-rayed samples can be attributed to the higher energy content of X-rays. Generally, the antioxidant capacity of rose oil was comparable with that of α -tocopherol. Therefore, rose oil can enhance the oxidative stability of irradiated SBO.

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