Turkish Journal of Agriculture - Food Science and Technology, 10(12): 2628-2634, 2022
DOI: https://doi.org/10.24925/turjaf.v10i12.2628-2634.5511

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Physicochemical Investigation of Some Oil Emulsions Oxidized by UV-B Radiation

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ARTICLE INFO

Research Article

Received : 14/09/2022
Accepted : 29/11/2022

Keywords:
Canola oil
UV-B radiation
O/W emulsion
Lipid peroxidation
Fatty acid composition

ABSTRACT

In this study, the effects of UV B (50 µW/cm^2) radiation at 306 nm on oil-in-water emulsions (O/W) using canola oil, soybean oil and linoleic acid were investigated. The oxidation rates of emulsions incubated at pH 7.0 and 37°C in the presence and absence of Cu (II) ions were determined by using iron (III) thiocyanate and thiobarbituric acid methods for the determination of primary and secondary products, respectively. The UV B-induced oxidation rates followed the order LA / Cu (II) > LA > Canola Oil / Cu (II) > Canola Oil > Soybean Oil / Cu (II) > Soybean Oil for both crops. Simultaneously, structural studies were performed using gas chromatography mass spectrometry (GC-MS). It was found that 18-carbon polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) contents have an important role in canola and soybean oil oxidation. The unsaturated fatty acid contents of small carbon numbers increased for each emulsion sample, while the oxidation of fatty acid changes did not follow a regular order.

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Introduction

Formulated food products often contain a lipid phase dispersed in an aqueous medium, which we define as oil-in-water (O / W) emulsions. Food emulsions are subjected to a wide range of physical-chemical processes, starting from their production to deconstruction in the digestive tract (Claire et al., 2014). Most changes in lipids in the emulsion depend on their physicochemical properties; in particular, the degree of unsaturation of fatty acids and the presence of oxidation initiators; exposure to oxygen and light, metallic ions or high temperature (Xu et al., 2012; Roman et al., 2013; Khanum and Thevanayagam, 2017). Especially ultraviolet B (UV B) radiation, together with oxygen and air pollutants, can lead to the production of hydroxyl radicals in the initiation of lipid peroxidation and can seriously damage biological structures (Trommera et al., 2001; Sourivonga et al., 2007; Brenneisen et al.,1998). Under these conditions, especially the chemically reactive components of lipids such as polyunsaturated fatty acids (PUFAs) are prone to oxidation. Edible oils contain both saturated and unsaturated fatty acids linked to glycerol as triacylglycerols. Unsaturated fatty acids (UFAs) contain allyl groups which are highly sensitive to free radical reactions and decompose in the presence of oxygen even at low temperatures (Xu et al., 2017). For example, oxidation of water-emulsified linoleic and linolenic acids yields a significant amount of unsaturated aldehydes (Baker et al., 1966; Roman et al., 2013). Currently, peroxide values (PV) determination, thiobarbituric acid reactive substances (TBARS) analysis and chromatography are the most common methods used to elucidate the classical chain reaction mechanism of lipid oxidation (Waraho et al., 2011; Chen et al., 2018).

The difference of this study from other fatty acid composition studies is that the fatty acid changes occurring in the emulsion during oxidation are determined simultaneously with lipid peroxidation kinetic data. Therefore, in this study, it was aimed to investigate fatty acids formed in water emulsions of canola oil, soybean oil and linoleic acid, in UV-B radiation and in the presence / absence of ionized Cu (II) by GC-MS structural analysis.

Table 1. Chromatographic assay of canola and soybean oil as methyl esters

Fatty acid	Fatty acid content ± SD (g per 100g)	
	Canola Oil	Soybean Oil
Palmitic acid, C16:0	4.38	6.27
Stearic acid, C18:0	1.92	1.79
Oleic acid, C18:1	61.02	16.61
Linoleic acid, C18:2	20.53	68.93
Linolenic acid, C18:3	8.72	6.58

Table 2. Rate constants of primary and secondary product formation of Cu (II) and UV B induced lipid oxidation.

Oil Species	Fe(III)SCN Method		TBARS Method	
Oil Species	$k_1 \pm S_k (hour^{-1}) \times 10^{-2}$	r^2	$k_2 \pm S_k (hour^{-1}) \times 10^{-2}$	r^2
Canola Oil / Cu (II)	0.74±0.09	0.945	0.25±0.00	0.998
Canola Oil	0.54±0.04	0.979	0.24±0.01	0.986
Soybean Oil / Cu (II)	0.51±0.06	0.939	0.20±0.02	0.961
Soybean Oil	0.48±0.02	0.994	0.18±0.01	0.980
LA / Cu (II)	0.98±0.07	0.981	0.56±0.04	0.976
LA	0.89±0.07	0.975	0.54±0.06	0.953

k_1: pseudo-first order rate constant with respect to hydroperoxides formation (measured by Fe (III)SCN method). k_2: pseudo-first order rate constant with respect to malondialdehyde formation (measured by TBARS method).

Materials and Methods

Standards, Instruments and Light Source

Canola oil and soybean oil used in this study were purchased from Sigma-Aldrich Co. LLC (Steinheim, Germany). Linoleic acid used as standard (95 %) were obtained from Alfa Aesar (Thermo Fisher GmbH, Germany). The absorption measurements were recorded with a SHIMADZU UVM-1240 UV-Visible spectrophotometer (Shimadzu Corp., Kyoto, Japan manufacture). All the experiments were carried out at 37°C by means of a thermostated system (±0.1°C) that contained an immersion circulator (NUVE BM 30 Circulation Water Bank, Ankara, Türkiye).

A 20 W narrowband UV B source capable of emitting between 290 nm and 315 nm (peak 306 nm) was purchased from Philips (Koninklijke Philips Electronics N.V., Türkiye Dealer). UV intensity was measured by UV digital radiometer (Model 5.0/UVA+B, Spectral Response: 280-400 nm,Sensor:GaAsP Photodiode,Solartech Inc.,USA). To obtain the value of the dose response associated with the effect of UV radiation, the oils were irradiated to UV radiation for a distance of 40 cm without visible beam in an incubator with a UV lamp at 37 °C. The intensity of UV lamp was 50 $\mu W/cm^2$ and the irradiated energy was controlled with exposure time using following equation;

$$Joule = Watt \times Seconds$$

Lipid Peroxidation Assay by UV B Radiation

Peroxidative process was initiated by ultraviolet radiation (UV B) with a wavelength range 290-315 nm exposed for 300 min. Before starting the peroxidation process, GC-MS chromatographic test results of used canola and soybean oils are given in Table 1. Accordingly, the saturated and unsaturated fat ratios of both oils were found to be almost the same. The amount of oleic acid was higher in canola oil and linoleic acid was higher in soybean oil.

Preparation of O/W Emulsions

Span 80 and Tween 80 emulsifier mixture was used for the O/W emulsions. The hydrophilic liphophilic balance (HLB) value of this mixture was fixed at 10 for all oil emulsions. 0.2 g oil was added to a 40 mL volumetric flask. Stock solution (2 mL) was added as an emulsifier (HLB: 10) (Hassan, 2015). $CuCl_2$ (0.01 M) was used as a chelator for the catalytic oxidation of oils and phosphate buffer (0.2 mol L^{-1}, pH 7) solution was used for the incubation medium. The emulsion was then homogenised in the brand homeogeniser (VELP-OV5, Homogenizator) at 3500 rpm.

Analysis of Hydroperoxides Value (HV)

The ferric thiocyanate (Fe (III) –SCN) method was used to detect the primary oxidation products (hydroperoxides) in the O/W emulsion system. The absorbance of the Fe (III) -SCN complex was measured at 500 nm, and after adding ethanol (4.7 mL, 75 % v/v), ammonium thiocyanate (0.1 mL, 30 % w/v) and ferrous chloride (0.1 mL of 0.02 M in 3.5 % v/v HCl) (Yıldoğan-Beker et al., 2011).

Analysis of Malondialdehide (MDA)

The malondialdehyde (MDA) content of the oil emulsions were measured as a secondary product (i.e., aldehydes/ketones, represented by MDA) of lipid peroxidation using the method of (Ohkawa et al., 1979) with a slight modification. Briefly, the oxidation degree was measured by sequentially adding water (2.65 mL), trichloroacetic acid (0.15 mL, 2.8 % w/v), sample solution (0.1 mL) and thiobarbituric acid (TBA; 0.1 M) at pH 3.5. The mixture was then incubated at 100 °C for 15 min. Later the absorbance of pink-colored MDA-TBA adduct was measured at the absorption maximum wavelength of 532 nm (Bakır et al., 2014).

Determination of Peroxidation Rate

Canola and soybean oil oxidation was examined in terms of primary and secondary products using the pseudo-first order equation previously given by (Yıldoğan-Beker et al., 2011).

$$\ln(1-A_t)/A_t = \ln(1-A_0)/A_0 - k.t \quad (1)$$

A_0 is absorbance at baseline and A_t is absorbance at time *t*, which is proportional to the total concentration of primer and seconder products (A_{500} nm or A_{532} nm). The

calculated results were shown by the rate constant of formation of primary products (k_1) and the rate constant of formation of secondary compounds (k_2).

Analysis of Fatty Acid Composition

Canola oil, soybean oil and linoleic acid oxidation process were performed by applying GS-MS analysis to emulsion samples taken at certain time intervals. For this purpose, hexane extraction was applied to the emulsion samples. Fatty acid methyl ester analyzes (FAME) were applied to the obtained hexane phases according to the IUPAC standard method (International Union of Pure and Applied Chemistry, 1992). GC-MS measurements were performed using a Shimadzu GC-MS QP 2010 ULTRA instrument on a RTX-5MS Capillary column (30m; 0.25mm; 0.25μm) using an ion source temperature of 200°C.

Statistical Analysis

Descriptive statistical analyses for calculating the standard error of the mean were performed using Microcal Origin 8.0 (Origin Lab Corp., Northampton, MA).

Results and Discussion

Lipid oxidation can occur rapidly in emulsions due to the large surface areas that facilitate the interaction between water-soluble proxidants and lipids (Xu et al., 2012). In order to elucidate the oxidation mechanism, it is important to determine the secondary fatty acids by chromotographic methods as well as colorimetric methods in which conjugated dienes, hydroperoxides and malondialdehydes are determined.

Evaluation of Kinetic Parameters for Primary and Secondary Oxidation Product Formation

In this study, the effects of UV B (50 μW/cm^2) radiation on lipid peroxidation in oil emulsions (O / W) in the presence and absence of ionized Cu (II) were investigated. UV B-induced oxidation of water-emulsified canola oil, soybean oil and linoleic acids at pH 7.0 and 37°C was determined using iron (III) thiocyanate and thiobarbituric acid methods for the determination of hydroperoxides and malondialdehyde respectively. The absorbances obtained were read against time and given in Figure 1 for canola oil, soybean oil and linoleic acid. Accordingly, in the environment with UV B + Cu (II) initiator, after 300 min, canola and soybean oil have higher absorbance values in terms of both primary and secondary product formation than UV B initiator medium. Likewise, the absorbance values obtained in Cu addition medium in linoleic acid started with higher sigmoidal curves and ended with higher absorbance.

The formation rates of the primary and secondary oxidation products for the lipid oxidation system with Cu (II) and UV B initiator are given in Table 2. In the UV B initiator system, the pseudo-first order rate constants obtained for both primary oxidation products and secondary oxidation products followed, LA /Cu (II) > LA >Canola Oil / Cu (II)> Canola Oil> Soybean Oil / Cu (II)> Soybean Oil.

Evaluation of GC-MS Chromatographic Analysis of Fatty Acids During Peroxidation

In addition to spectroscopic analyzes, chromatographic GC-MS, methyl ester assays are a powerful tool to characterize oxygenated fatty acids derived from lipid oxidation. This study focuses on the applications of GC-MS in structural analysis related to possible structures during UV B induced oxidation of lipid emulsion samples. GC-MS analysis of hexane extracts of samples (0.1 mL) taken during oxidation of both UV B and UV B / Cu initiators of canola oil, soybean oil and linoleic acid emulsions were investigated. Fatty acid changes over time for each lipid emulsion sample were given in Figure 2 for canola oil, soybean oil and linoleic acid. Accordingly, canola and soybean oil emulsions in both initiator systems, almost all of the total saturated fatty acids (SFA) Hexadecanoic acid, methyl ester (C16: 0, Palmitic acid, methyl ester) and methyl stearate (C18: 0, Stearic acid, methyl ester) and very low changes in fatty acid percentages during oxidation were detected. Generally, UFAs in oils are mostly composed of MUFAs and PUFAs were found to be more susceptible to oxidation than MUFAs. (Chena et al., 2018).

In this study, 18 carbon PUFA and MUFA were found to have an important role in canola and soybean oil oxidation. The content of trans fatty acid (TFA) consists of 18 carbon PUFAs (11-octadecenoic acid (trans-vaccenic acid, C18:1n7), 9-octadecenoic acid (elaidic acid, C18:1w9) and 10-trans, 12-cis-octadecadienoic acid (C18:2w6,8)).

The total SFA remained virtually constant in all three-emulsion media during oxidation with UV B initiator alone. During canola oil oxidation in the UV B initiator medium, the total MUFA remained unchanged while the total TFA content increased (Figure 2 (1A)). 9,12-octadecadienoic acid (linoleic acid, C18:2w6) (0.39-17.77g / 100g oil) constitutes the majority of total PUFAs and decreased after 90 minutes of oxidation. In the UV B initiation oxidation of canola oil, trans-vaccenic acid (3,15-71,84g / 100g oil) and elaidic acid (25,29-55,90 g / 100g oil) trans fatty acids were frequently observed. At this stage, cis-, trans-conjugated linoleic acid (CLA) isomers were thought to form trans-, trans-CLA isomers which remained more stable in photoisomerization (Jain and Proctor, 2007a; Jain and Proctor, 2007b). These results showed that PUFAs are more effective in terms of trans fat formation in this system. Secondary product (SP) formations were lower than UV B / Cu induced environment.

In canola oil oxidation in Cu added UV B medium, trans-vaccenic acid (2.16-50.21 g / 100g oil) and elaidic acid (15.96-27) 87 g / 100 g oil) formed the majority of trans fats and showed no linear change (Figure 2 (1B)). Towards the end of the oxidation time, 10-trans, 12-cis-octadecadienoic acid (C18:2w6,8) (5.24-7.70 g / 100g oil) and trans-13-octadecenoic acid (C18:1w5) (5,27 g/100g oil) compounds were observed. Linoleic acid (9.64-16.66 g / 100 g oil) and 4,7,10,13 16,19-docosahexaenoic acid (C22:6) (0,11-17,18 g / 100g fat) gradually decreased during the oxidation process and converted to many fatty acids between C18-22.

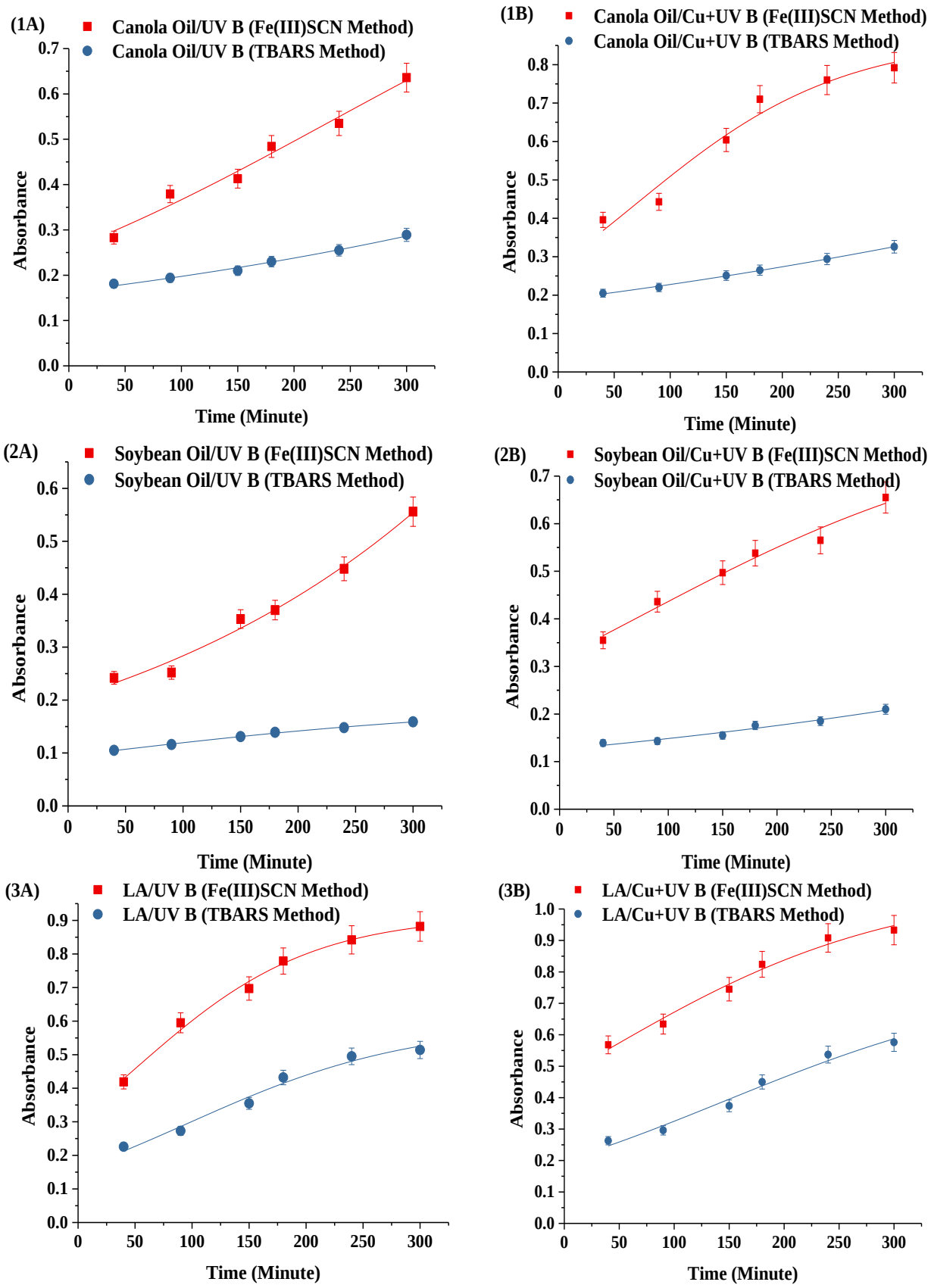

Figure 1. The absorbance–time graph for the canola oil (1), soybean oil (2) and linoleic acid (3) oxidation with (A) UV B and (B) UV B + Cu (II) initiator (measurements made with Fe(III)SCN (500 nm) and TBARS (532nm))

In addition to short-chain oxygenated fatty acids that were previously produced from the cleavage of alkoxy radicals in oxidized lipids, the formation of oxygenated fatty acids of the original chain length was also defined as lipid oxidation products (Berdeaux et al., 2002). Cholesterol (1.43-35.38 g / 100g fat) was observed as the secondary product in this medium.

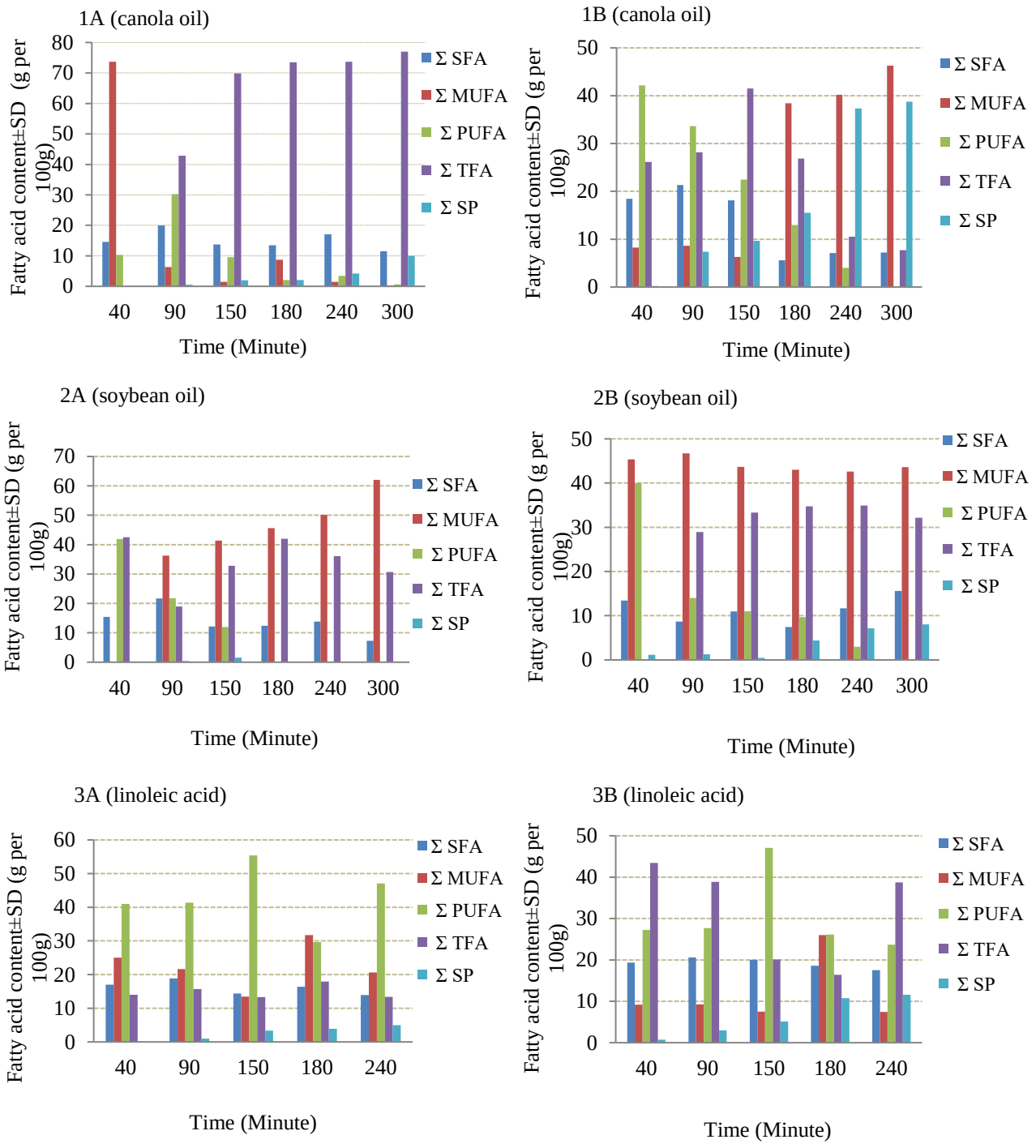

Figure 2. Change of fatty acid contents of during canola oil (1), soybean oil (2) and linoleic acid (3) oxidation with (A) UV B and (B) UV B + Cu(II) initiator

When exposed to light, oxygen and heat, it can produce cholesterol oxidation products such as cholesterol, 7-ketocholesterol, 7-hydroxycholesterol and 5,6-epoxycholesterol (Yen et al., 2010). Aldehyde species such as 8-Hexadecenal,14-methyl (0.75-3.20 g / 100g oil) were formed secondary.

During the oxidation of soybean oil in UV B induced environment, total MUFA values increased regularly while total PUFA values decreased rapidly (Figure 2 (2A)). Linoleic acid (41.58 g / 100g oil) was initially found to be the most abundant PUFA. The increase in TFA showed a regular change up to 180th minute with the decrease of total PUFA values and a decrease after this minute. In contrast, the value of MUFA consists of 9-octadecenoic acid (oleic acid, C18:1w9) (0.11 to 31.56 g / 100 g oil) compound and then increased with the formation of cis-11-octadecenoic acid (cis-vaccenic acid, C18:1n7) (41.38-62.01 g / 100g oil) compound up to 90 minutes. This result suggested that total MUFA increase was related to trans fatty acid exchange. Initially, 9,12-octadecadienoic acid (linolelaidic acid, C18:2w6,9) (19,91 g/100g oil) and elaidic acid (42,51 g/100g oil) trans fats were formed, whereas 10-trans,12-cis-octadecadienoic acid (32,78-41,99 g/100g oil) formed at a higher rate between 150-240

minutes. Total secondary product (SP) formation was observed at minimum.

Secondary product increase was observed in soybean oil with UV B / Cu induced trans oil change (Figure 2 (2B)). At the 40th minute of oxidation, linoleic acid (39.92 g / 100g oil) amounted to the majority of PUFA and decreased over time. Decreasing PUFA over time may increase secondary product formation. Oils with a high linoleic acid content contain high amounts of saturated aldehyde. Soybean oil was found to contain volatile flavor ingredients such as pentanal, hexanal, benzaldehyde and 1-pentanol. These volatile components are important oxidative degradation products of oleic acid and linoleic acid (Xu et al., 2017). For soybean oil, Cycloheptasiloxane and Octasiloxane varieties were formed in this medium and increased the amount of secondary products. cis-Vaccenic acid (42.58-46.72 g / 100g oil), the MUFA value remained high over time and almost unchanged. The increase of the trans-fat ratio by oxidation can be considered as the increase in the amount of 10-trans, 12-cis-octadecadienoic acid (17.71-33.31 g / 100g oil) compound in each interval.

Autoxidation of linoleic acid involves hydrogen abstraction on allyl C-11 and formation of a pentadienyl radical. This radical reacts with oxygen to produce a mixture of conjugated 9- and 13-diene hydroperoxides (Shahidi, 2005). Total MUFA and PUFA values did not show a linear increase or decrease while linoleic acid was oxidized in UV B medium (Figure 2 (3A)). In this medium, PUFA composed of linoleic acid (8.3-24.91 g / 100g oil) and 4,7,10,13,16,19-docosahexaenoic acid (14.56-23.90 g / 100 g oil) compounds. The values of these two compounds changed during oxidation. The majority of the MUFA value was obtained by the presence of oleic acid (13.46-26.16 g / 100g oil). The amount of TFA was completely changed by a change of linolelaidic acid (10.57-17.96 g / 100g oil). This result confirms that PUFAs are more effective in TFA formation in linoleic acid oxidation.

In the UV B / Cu induced medium, linoleic acid trans-fat change first decreased and then increased slowly, PUFA values showed the opposite situation (Figure 2 (3B)). Linolenic acid (0.19-0.57 g / 100g oil) was detected in all samples in total PUFA. Oxidative degradation of linolenic acid is faster than oxidative degradation of linoleic acid and oleic acid, and linolenic acid is an important source of TFA in edible oils (Morales et al.,1997).

Most of the PUFAs in this medium are linoleic acid (6,46-16,20 g / 100g fat) and 4,7,10,13,16,19-docosahexaenoic acid (C22: 6w3,6,9,12,15,18) (10.51-17.41 g / 100 g oil). The amount of trans fat is greater than oxidation with UV B initiator alone and 10-trans, 12-cis-octadecadienoic acid and elaidic acid formed the trans-fat ratio. Alkaline species such as octadecene and hexadecene, alcohol species such as docosanol and many siloxane species have occurred.

Conclusions

Oxidation of polyunsaturated fatty acids leads to the formation of a conjugated dienoic structure that is maintained along the lipid oxidation chain, as is the continuous exchange of conjugated dienes. In all oil types examined, PUFAs showed more variation in UV B / Cu induced environments than MUFAs, and SP formations were faster than UV B induced environments. The results showed that the degree of unsaturation of the oils tested was the dominant parameter affecting the progression of oxidation kinetics.

Acknowledgements

We are grateful to Filmak Industrial Products Industry and Trade Limited for UV B device support. “This study was published as an abstract paper in 2nd International Eurasian Conference on Biological and Chemical Sciences hold from June 28 to 29, 2019, in Ankara, Türkiye.”

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