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ORIGINAL PAPER

Distribution of Triacylglycerols and Fatty Acids in Soybean Oil with Thermal Oxidation and Methylene Blue Photosensitization

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Abstract Profiles of triacylglycerols (TAG) and fatty acids were compared in soybean oil thermally oxidized at 180 °C for 60 min or methylene blue photosensitized for 10 h. Headspace oxygen in thermally oxidized and photosensitized soybean oil decreased significantly ($p < 0.05$) as oxidation time increased. Relative contents of linoleic and linolenic acids decreased and those of oleic acid increased during oxidation. In both thermal and photosensitized oxidation, TAG with lower than 44 equivalent carbon number including dilinoleoyllinolenoylglycerol (LLLn, 40), trilinolein (LLL, 42), oleoyllinoleoyllinolenoylglycerol (OLLn, 42), dilinoleoyloleoylglycerol (LLO, 44), and dilinoleoylpalmitoylglycerol (PLL, 44) significantly decreased, while those with dioleoyllinoleoylglycerol (OOL, 46) increased significantly in relative peak areas ($p < 0.05$). Photosensitized oxidation decreased TAG containing linoleic and linolenic acids significantly faster than thermal oxidation in soybean oil ($p < 0.05$), which may be due to the singlet oxygen reaction. Photosensitized soybean oils can be differentiated from thermally oxidized samples using the distributions of TAG by principal component analysis.

Keywords Triacylglycerols · Fatty acids · Autoxidation · Methylene blue photosensitization · Soybean oil

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Introduction

Lipid oxidation is one of representative chemical reactions in lipid-rich foods during processing and storage. Oxidized lipids can cause the sensory quality and nutritional values to deteriorate in foods and decrease the consumers' acceptance severely. Mechanisms of lipid oxidation including autoxidation, singlet oxygen oxidation, lipoxygenase-related oxidation, and deep-fat frying have been suggested to understand the oxidation process in foods [1, 2].

Autoxidation is a free radical chain reaction through initiation, propagation, and termination steps [1]. At higher temperature such as deep-fat frying, not only oxidation but also hydrolysis, pyrolysis, and polymerization can occur resulting in physicochemical changes in oils and fried foods [2]. Photosensitized oxidation is one of the important oxidation pathways for foods containing photosensitizers and stored under light irradiation. Photosensitizers, including chlorophylls, riboflavin, and methylene blue, can be excited by irradiation of visible light. The excited sensitizers can generate new radicals by abstracting a hydrogen atom or an electron through a type I pathway or convert triplet oxygen into singlet oxygen through a type II pathway [3]. Several studies have reported that photosensitization can accelerate the chemical reactions in model and real food systems. Formation of sun-light flavor in riboflavin sensitized milk [4], oxidized volatile formation in chlorophyll sensitized lard [5] and linoleic acid [6], and the decrease of phytochemicals like isoflavones in a riboflavin model system [7] are some examples of detrimental influence of photosensitized oxidation on foods.

Triacylglycerol (TAG) is composed of one glycerol backbone and three ester linked fatty acids. TAG are the predominant components of dietary fats and oils and the profiles of TAG influence the physicochemical properties of

oil-containing foods greatly. Polymorphism of fats and oils are physical properties influenced by the distribution of TAG and mode of sample preparation [8]. Therefore, analysis of TAG profiles is one of the critical steps to understand the physicochemical properties of edible fats and oils.

Profiles and positions of TAG can affect the oxidative stability of edible oils [9–11]. Hoshina et al. [9] reported that TAG mixtures of unsaturated fatty acids and saturated fatty acids (2:1, mol/mol) such as tripalmitin (PPP, P:palmitic acid):trilinolein (LLL, L:linoleic acid) were more susceptible to thermal oxidation at 150 and 180 °C than a 1:1 ratio of PPP: dilinoleoylpalmitoylglycerol (PLL) or dipalmitoyllinoleoylglycerol (PPL) only. Endo et al. [10] reported that the position of eicosapentaenoic acid in synthetic TAG affected the autoxidation rate. Miyashita et al. [11] showed that position of linolenic (Ln) and linoleic (L) acids influenced the oxidative stability in TAG and 1,2-dilinolenoyl-3-linoleoylglycerol (LnLnL) was oxidized faster than 1,3-dilinolenoyl-2-linoleoylglycerol (LnLLn). Also, oxidation products including monohydroperoxides, epoxides or bishydroperoxides were identified from triolein (OOO), LLL and trilinolein (LnLnLn) treated with autoxidation [12].

Extensive studies have been conducted to understand the mechanisms of lipid oxidation in real foods or model systems. However, reports on the profile changes of TAG and fatty acids in soybean oil treated with photosensitized oxidation are limited in the literature although soybean oil is mainly composed of TAG. Also, pigments possessing a photosensitizing ability are present in commercially available refined vegetable oils [13], which are displayed in markets under visible light irradiation.

The objectives of this study were to determine the distribution of TAG and fatty acids in soybean oil treated with thermal oxidation at 180 °C and methylene blue photosensitized oxidation and to compare the profiles of TAG using principal component analysis (PCA).

Materials and Methods

Materials

Soybean oil was purchased from a local grocery market (Seoul, Korea). Mixtures of 5 TAG including tricaprylin (CyCyCy), tricaprin (CCC), trilaurin (LaLaLa), trimyristin (MMM), and PPP and 10 standard TAG including tristearin (SSS), OOO, LLL, LnLnLn, 1,2-dilinoleoyl-3-palmitoylglycerol (LLP), 1,2-dioleoyl-3-palmitoylglycerol (OOP), 1,2-dioleoyl-3-stearoylglycerol (OOS), 1-palmitoyl-2-oleoyl-3-linoleoylglycerol (POL), 1,3-dipalmitoyl-2-oleoylglycerol (POP), and 1,2-distearoyl-3-oleoylglycerol (SSO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). A mixture of standard fatty acid methyl esters (FAME) were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade solvents were purchased from Fisher Scientific (Fairlawn, NJ, USA).

Sample Preparation

Soybean oil was treated to remove impurities according to the method of Lee et al. [14]. Briefly, soybean oil was purified using a glass column packed with activated silicic acid (5 g), powdered sugar and celite mixture (4:1), activated charcoal and celite mixture (4:1), and activated silicic acid (5 g) from top to bottom.

Four grams of purified soybean oil was put in a 10-mL sample bottle and sealed with a rubber septum and an aluminum cap. Samples for thermal oxidation were put in a convection oven (Win Science, Seoul, Korea) at 180 °C for 60 min. Methylene blue was dissolved in acetone and added to 4 g of purified soybean oil to make 0.234 μmol/g oil in 10-mL bottles. Solvent was removed under a nitrogen gas flow and sample bottles were sealed air-tight with rubber septa and aluminum caps. Samples with methylene blue were placed in a light box with 1,333 Lux for photosensitization at room temperature. Samples for the dark condition were prepared by wrapping bottles in an aluminum foil. Soybean oil with methylene blue in the dark, with methylene blue under light, and with thermal oxidation were designated as SMD, SML, and STO, respectively. SMD and SML were sampled at 0, 1, 2, 4, 8, and 10 h and STO was sampled at 0, 10, 30, 40, 50, and 60 min, respectively. Samples were prepared in triplicate at each sampling time.

Headspace Oxygen Analysis

The degree of oxidation was determined by the depletion of headspace oxygen in air-tight samples containing soybean oil. The headspace oxygen in air-tight sample bottles was analyzed according to Lee et al. [5]. Twenty-five milliliters of headspace gas was removed from a sample bottle by an air-tight syringe and oxygen contents were determined using GC-a thermal conductivity detector (TCD). A Hewlett-Packard 5890II GC (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a MS-5A 60/80 packed column (3.0 m × 2 mm ID, Restek Ltd., USA) and a TCD from Agilent Technologies (Palo Alto, CA, USA) was used. The flow rate pressure of the helium gas was 30 psi. The temperatures of the oven, injector, and a thermal conductivity detector were 60, 180, and 180 °C, respectively.

Fatty Acid Analysis

Fatty acids were derivatized to FAME using BF_3/MeOH (14% boron trifluoride) according to AOAC method 969.33 [15]. The FAME were analyzed by a Hewlett-Packard

5890II gas chromatograph (Agilent Technologies) with a flame ionization detector (FID), and a DB-23 (60 m × 0.32 mm ID, 0.25 μm film) from J&W Scientific (Folsom, CA, USA). The oven temperature started at 100 °C for 1 min, increased to 195 °C at 15 °C/min, to 210 C at 1 °C/min, and to 240 °C at 5 °C/min and held at 240 °C for 7.5 min. The temperatures of both injector and detector were 260 °C. The flow rate of the helium carrier gas was 1.1 mL/min, the injection volume was 1 μL, and the split ratio was 1:50. Peaks of GC chromatograms were identified comparing the retention times of a mixture of standard FAME (Sigma–Aldrich).

Triacylglycerol Analysis by HPLC-ELSD

TAG were analyzed using a HPLC (Jasco Pu-2089 plus, JASCO International Co., Ltd, Tokyo, Japan) equipped with an evaporative light scattering detector (ELSD) (SofTA^{TM} ELSD-400, SofTA, Colorado, USA). The stationary phase was a Nomura ^{TM} Develosil C30-UG-5 (300 mm × 3.9 mm I.D.) column and the mobile phase was a mixture of acetonitrile (solvent A) and dichloromethane (solvent B) at a flow rate of 1.0 mL/min with gradient: increase of solvent B from 30 to 70% for 60 min, staying for 10 min, and then re-equilibration of solvent B at 30% for 50 min. Temperature of column oven, drift chamber, and spray chamber were 25, 60, and 30 °C, respectively. Injection volume of sample was 10 μL [16].

TAG from HPLC-ELSD were identified using the retention times of standard TAG and previous reports [16–18]. Standard TAG were properly diluted with dichloromethane and analyzed under the same HPLC conditions.

Statistical Analysis

Results of TAG and headspace oxygen contents were statistically analyzed by ANOVA and Duncan's multiple range test using SPSS software program (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered significant. PCA for the distribution of TAG was conducted using Microsoft Excel software program.

Results and Discussion

Headspace Oxygen Analysis

Headspace oxygen contents in soybean oil with thermal or methylene blue photosensitized oxidation are shown in Fig. 1. Headspace oxygen in thermally oxidized and methylene blue photosensitized soybean oil decreased with the regression equations of $y = -0.219x + 21.316$ ($R^2 = 0.968$) and $y = -1.225x + 20.428$ ($R^2 = 0.986$),

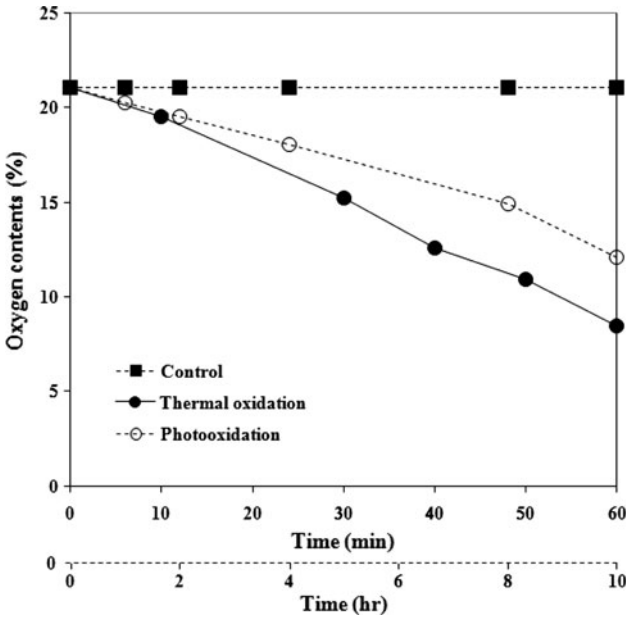

Fig. 1 Changes of headspace oxygen contents in soybean oil with thermal oxidation and photosensitized oxidation. The units of the *X*-axes for thermal oxidation and photosensitization are minutes (*straight line*) and hours (*dotted line*), respectively

respectively, where y is the oxygen contents in percent (%) and x is oxidation time. The unit of x is 'minute' for thermal oxidation and 'hour' for photosensitized oxidation, respectively. The headspace oxygen of samples in the dark were not significantly different for 10 h at room temperature ($p > 0.05$). Headspace oxygen contents is one of the representative methods for determining the degree of oxidation and singlet oxygen quenching mechanisms and kinetics [5, 19, 20]. Quenching mechanisms and kinetics of antioxidants including α-tocopherol, riboflavin, and β-carotene for singlet oxygen were determined through the depletion of headspace oxygen contents [19, 20]. The changes of headspace oxygen were compared in elaidic *trans*-fatty acid and oleic *cis*-fatty acid under methylene blue photosensitization and thermal autoxidation [21].

Headspace oxygen contents of soybean oil with methylene blue photosensitization for 10 h at room temperature and thermal oxidation at 180 C for 40 min were 12.10 and 12.60%, respectively, which implies that these two samples underwent similar degree of oxidation based on the headspace oxygen analysis. Profiles of TAG and fatty acids were compared between 40 min thermal oxidized and 10 h photosensitized soybean oil samples.

Fatty Acid Analysis in Soybean Oil with Thermal Oxidation and Photosensitization

Changes of selected major fatty acids in SO treated with thermal oxidation and methylene blue photosensitization

are shown in Table 1. Major fatty acids in soybean oils before oxidation were palmitic acid (16:0, 10.85%), stearic acid (18:0, 4.22%), oleic acid (18:1, 22.36%), linoleic acid (18:2, 53.34%) and linolenic acid (18:3, 6.95%). As thermal oxidation time increased to 60 min, the relative percentage of linoleic and linolenic acids decreased by 0.87 and 0.65%, respectively, while those of oleic acid increased by 1.39%. *trans*-Fatty acids, which were not detected before thermal oxidation, were observed in all the thermally oxidized samples (Table 1). In case of 10 h-methylene blue photosensitization, relative percentage of linoleic and linolenic acids decreased by 0.80 and 0.71%, respectively, while those of oleic acid increased by 1.43%. *trans*-Fatty acid of 18:2 was first detected in 8 h treated samples while *trans* 18:3 fatty acid was observed from samples of 1 h photosensitization (Table 1). Although relative contents of linoleic acid were about 7.67 times more than those of linolenic acid, singlet oxygen can react with linolenic acid faster than linoleic acids and generated detectable amounts of *trans* 18:3 fatty acids. Distributions of fatty acids between soybean oil treated with 10 h photosensitization and 40 min thermal oxidation were similar to each other (Table 1).

Generally, lipid oxidation decrease the contents of polyunsaturated fatty acids faster than of monounsaturated fatty acids and the relative percentages of monounsaturated fatty acids and saturated fatty acids increase compared to those of polyunsaturated fatty acids. The reaction rates of singlet oxygen with oleate, linoleate, and linolenate are 30,000, 1,450, and 909 times faster than triplet oxygen, respectively [1]. The large changes of linoleic and linolenic acids in photosensitization are due to the fast reaction of singlet oxygen with double bonds of unsaturated fatty acids.

Triacylglycerol Analysis from Oxidized Soybean Oil

HPLC chromatograms of TAG in soybean oil treated with thermal oxidation and methylene blue photosensitization are shown in Fig. 2. TAG were clearly separated and detected under current HPLC analytic condition using a C_{30} column and an ELSD. HPLC-ELSD has been used successfully to separate TAG in edible oils [16, 18, 22]. Cunha and Oliveira [18] compared TAG profiles of eight edible oils including soybean, sunflower, olive, and sesame oils using an ELSD and a C_{18} column by HPLC-ELSD.

Thermal oxidation and photosensitized oxidation caused great changes in peak areas of TAG in soybean oil (Fig. 2). Peak of dilinoleoyllinolenoylglycerol (LLLn) decreased apparently and peaks of OOO and POO increased noticeably compared to those in untreated soybean oil, which implies that significant changes of TAG occurred during oxidation.

Table 1 Relative percentage (%) of major fatty acids in soybean oil treated with thermal oxidation and photosensitization

Name	Area (%)										
	Soybean oil	STO[a]-10 min	STO-30 min	STO-40 min	STO-50 min	STO-60 min	SML-1 h	SML-2 h	SML-4 h	SML-8 h	SML-10 h
16:0	10.85 ± 0.01[b]	10.68 ± 0.00	10.73 ± 0.01	10.72 ± 0.01	10.74 ± 0.03	10.75 ± 0.01	10.56 ± 0.02	10.60 ± 0.03	10.57 ± 0.03	10.57 ± 0.03	10.59 ± 0.03
18:0	4.22 ± 0.01	3.98 ± 0.01	4.05 ± 0.00	4.03 ± 0.01	4.04 ± 0.01	4.04 ± 0.00	3.95 ± 0.01	3.93 ± 0.02	3.99 ± 0.01	3.98 ± 0.01	4.01 ± 0.01
18:1 *cis*	22.36 ± 0.00	23.62 ± 0.00	23.75 ± 0.02	23.70 ± 0.00	23.71 ± 0.02	23.75 ± 0.00	23.76 ± 0.05	23.73 ± 0.08	23.78 ± 0.05	23.72 ± 0.05	23.79 ± 0.05
18:2 *trans*	0.00 ± 0.00	0.27 ± 0.00	0.27 ± 0.00	0.27 ± 0.00	0.27 ± 0.00	0.28 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.00	0.33 ± 0.00
18:2 cis	53.34 ± 0.02	52.89 ± 0.01	52.57 ± 0.06	52.51 ± 0.01	52.59 ± 0.08	52.47 ± 0.01	52.94 ± 0.12	53.34 ± 0.18	52.87 ± 0.12	52.64 ± 0.12	52.54 ± 0.13
18:3 *trans*	0.00 ± 0.00	0.19 ± 0.00	0.29 ± 0.08	0.19 ± 0.00	0.29 ± 0.08	0.19 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
18:3n-3	6.95 ± 0.01	6.42 ± 0.00	6.12 ± 0.18	6.34 ± 0.00	6.13 ± 0.19	6.30 ± 0.00	6.32 ± 0.20	5.97 ± 0.33	6.31 ± 0.20	6.27 ± 0.20	6.24 ± 0.20
20:0	0.76 ± 0.01	0.70 ± 0.00	0.72 ± 0.00	0.73 ± 0.01	0.72 ± 0.01	0.72 ± 0.01	0.72 ± 0.00	0.71 ± 0.01	0.73 ± 0.00	0.73 ± 0.00	0.73 ± 0.01
20:1n-9	0.22 ± 0.00	0.23 ± 0.00	0.24 ± 0.00	0.24 ± 0.00	0.24 ± 0.00	0.23 ± 0.00	0.24 ± 0.00	0.24 ± 0.00	0.24 ± 0.00	0.24 ± 0.00	0.25 ± 0.00
22:0	1.02 ± 0.01	0.94 ± 0.00	0.97 ± 0.00	0.98 ± 0.00	0.97 ± 0.01	0.97 ± 0.00	0.99 ± 0.00	0.98 ± 0.01	1.00 ± 0.00	1.00 ± 0.00	1.01 ± 0.00
24:0	0.28 ± 0.00	0.28 ± 0.00	0.29 ± 0.00	0.30 ± 0.00	0.30 ± 0.00	0.30 ± 0.00	0.31 ± 0.00	0.31 ± 0.00	0.32 ± 0.00	0.32 ± 0.00	0.32 ± 0.00

[a] STO and SML are soybean oil samples with 180 °C thermal oxidation and with methylene blue under light irradiation, respectively

[b] Mean ± standard deviation ($n = 3$)

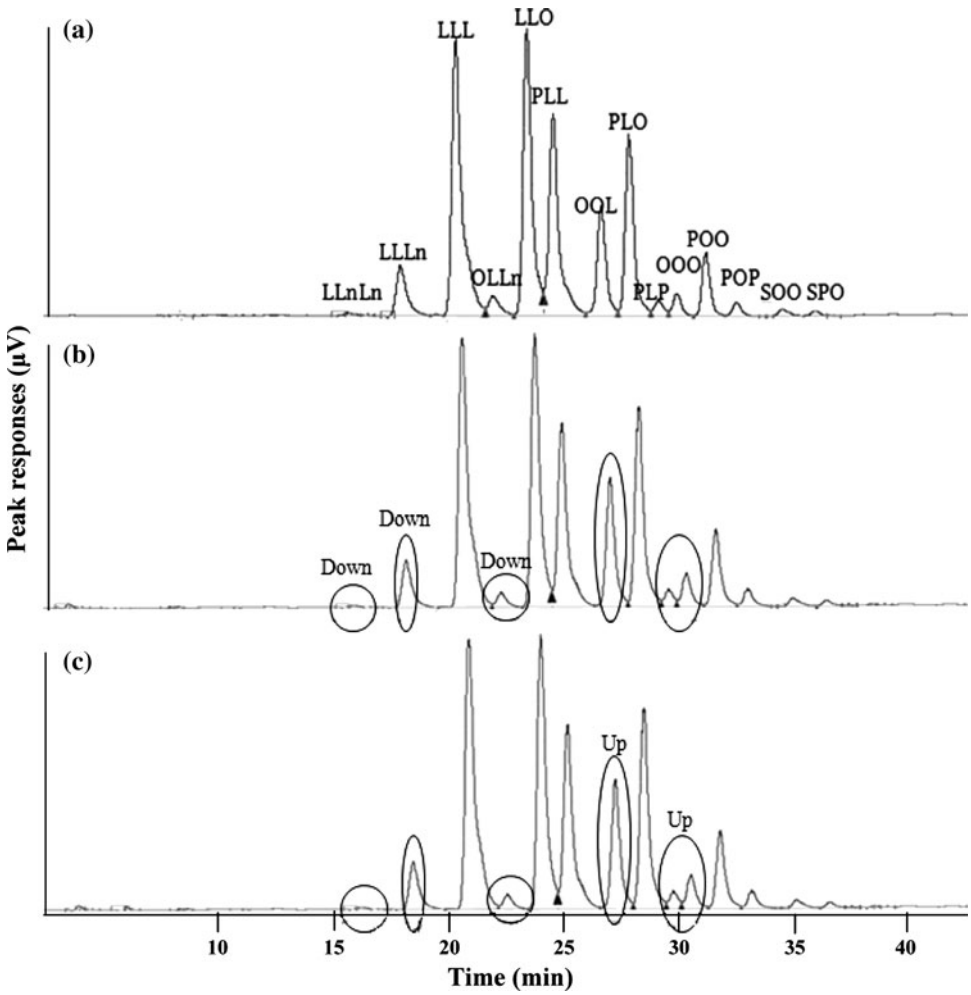

Fig. 2 HPLC chromatograms of triacylglycerols in soybean oil before treatment (**a**), after 40 min thermal oxidation (**b**), and after 10 h photosensitized oxidation (**c**). Terms of "*up*" and "*down*" indicate the increases or decreases of the TAG' relative peak areas compared to unoxidized samples, respectively

Distributions of TAG in soybean oil during thermal oxidation at 180 °C are shown in Table 2. Major TAG in untreated soybean oil were LLL, dilinoleoyloleoylglycerol (LLO), dilinoleoylpalmitoylglycerol (PLL), palmitoyllinoleoyloleoylglycerol (PLO), and dioleoyllinoleoylglycerol (OOL), and the relative peak areas of those TAG were 23.58, 22.10, 16.37, 14.74, and 8.42%, respectively. TAG containing linoleic acid were major TAG in soybean oil, which agrees with the results of fatty acid analysis. Cunha and Oliveira [18] reported that LLO (19.51%), POO (16.87%), OOL (15.61%), and PLO (14.40%) were major TAG in soybean oil in the order of abundance. Our results showed higher percentages of LLL and PLL and lower percentages of OOL and OOO in soybean oils than those of Cunha and Oliveira [18]. Considering the contents of oleic acid (22.36%), linoleic acid (53.34%), and linolenic acid (6.95%) in soybean oil, higher percentages of TAG containing linoleic acid such as LLL and PLL were expected in TAG analysis of soybean oil.

As thermal oxidation at 180 °C proceeded for 60 min, relative contents of LLLn, LLL, oleoyllinoleoyllinolenoylglycerol (OLLn), LLO, and PLL in soybean oil decreased significantly ($p < 0.05$). However, those of TAG including OOL and OOO increased significantly ($p < 0.05$). Interestingly, TAG with lower than 44 equivalent carbon number (ECN) including LLLn (40), LLL (42), OLLn (42), LLO (44), and PLL (44) decreased and those with higher than 46 ECN such as OOL (46) and OOO (48) increased in relative peak areas during thermal oxidation. Generally, lipid containing high linoleic and linolenic acids have lower oxidative stability than those with oleic and palmitic acids during thermal oxidation [1].

Profiles of TAG in soybean oil treated with methylene blue photosensitization at room temperature are shown in Table 2. Relative contents of LLLn, LLL, OLLn, LLO, and PLL in soybean oil decreased significantly ($p < 0.05$) while those of TAG including OOL and POO increased significantly ($p < 0.05$) in 10 h-photosensitized samples compared to untreated samples. Like thermally oxidized samples, TAG containing polyunsaturated fatty acids like linoleic and linolenic acids with lower than 44 ECN decreased significantly while those containing oleic and palmitic acids with higher than 46 ECN increased significantly during photosensitized oxidation ($p < 0.05$).

Table 2 Distribution of TAG in soybean oil during thermal oxidation at 180 °C and methylene blue photosensitized oxidation for 10 h at room temperature

TAG	Area (%)											
	RT[a]	Soybean oil[b]	STO-10 min	STO-30 min	STO-40 min	STO-50 min	STO-60 min	SML-1 h	SML-2 h	SML-4 h	SML-8 h	SML-10 h
LLnLn	17.34	0.23 ± 0.02[c] a[d]	0.25 ± 0.07a	0.21 ± 0.01a	0.20 ± 0.00a	0.20 ± 0.01a	0.19 ± 0.02a	0.21 ± 0.06ab	0.21 ± 0.01ab	0.19 ± 0.01ab	0.17 ± 0.02a	0.19 ± 0.01ab
LLLn	19.76	4.15 ± 0.13c	4.20 ± 0.41c	3.72 ± 0.03b	3.62 ± 0.15ab	3.58 ± 0.05ab	3.32 ± 0.10a	4.18 ± 0.09c	3.69 ± 0.09b	3.18 ± 0.01a	3.08 ± 0.26a	2.98 ± 0.05a
LLL	22.41	23.58 ± 0.35b	20.50 ± 1.11a	21.82 ± 0.12a	21.99 ± 0.23a	21.42 ± 1.79a	21.31 ± 0.09a	23.16 ± 0.75c	22.93 ± 0.29c	21.70 ± 0.40b	20.11 ± 0.16a	19.97 ± 0.35a
OLLn	24.26	1.84 ± 0.08c	1.68 ± 0.17bc	1.83 ± 0.13c	1.50 ± 0.25ab	1.36 ± 0.11a	1.36 ± 0.03a	1.43 ± 0.06c	1.26 ± 0.21bc	1.10 ± 0.10ab	0.97 ± 0.04a	1.04 ± 0.03a
LLO	25.81	22.10 ± 0.34c	20.09 ± 0.42b	18.55 ± 0.53a	19.74 ± 0.15ab	19.62 ± 1.62ab	19.74 ± 0.74ab	19.40 ± 0.32a	19.68 ± 0.56a	19.41 ± 0.04a	19.27 ± 0.06a	19.74 ± 0.15a
PLL	27.07	16.37 ± 0.33c	15.38 ± 0.38a	15.51 ± 0.32ab	15.99 ± 0.07bc	15.51 ± 0.47ab	15.17 ± 0.02a	14.45 ± 0.07a	14.15 ± 0.67a	15.60 ± 0.27b	14.05 ± 0.11a	14.07 ± 0.19a
OOL	29.35	8.42 ± 0.35a	9.41 ± 0.55b	9.43 ± 0.38b	8.98 ± 0.47ab	9.25 ± 0.50b	9.47 ± 0.15b	8.03 ± 0.15a	8.17 ± 0.35a	10.29 ± 0.11c	9.42 ± 0.41b	9.17 ± 0.13b
PLO	30.70	14.74 ± 0.18b	14.15 ± 0.44ab	13.73 ± 0.48a	13.88 ± 0.52ab	14.34 ± 0.05ab	14.65 ± 0.70b	12.51 ± 1.08a	13.79 ± 1.12ab	16.18 ± 0.21c	16.17 ± 0.46c	14.76 ± 0.75b
PLP	32.08	1.31 ± 0.09a	1.61 ± 0.24a	1.50 ± 0.41a	1.44 ± 0.33a	1.37 ± 0.33a	1.61 ± 0.43a	1.04 ± 0.04a	1.01 ± 0.01a	1.28 ± 0.19b	1.11 ± 0.08ab	1.21 ± 0.11ab
OOO	32.97	1.76 ± 0.14a	2.62 ± 0.33bc	2.18 ± 0.008ab	2.09 ± 0.13ab	2.38 ± 0.50bc	2.84 ± 0.43c	2.02 ± 0.05ab	2.52 ± 0.92ab	2.65 ± 0.23b	2.67 ± 0.17b	2.45 ± 0.11ab
POO	34.31	4.41 ± 0.27a	5.07 ± 0.46ab	4.61 ± 0.55a	4.51 ± 0.42a	4.88 ± 0.57a	5.82 ± 0.18b	4.73 ± 0.56ab	5.34 ± 0.40abc	5.83 ± 1.32bc	6.59 ± 0.29c	5.41 ± 0.50bc
POP	35.85	1.02 ± 0.07a	1.34 ± 0.35b	1.25 ± 0.30ab	1.02 ± 0.38a	1.03 ± 0.22a	1.38 ± 0.22ab	1.02 ± 0.06a	1.11 ± 0.08ab	2.82 ± 2.73ab	1.31 ± 0.09b	1.22 ± 0.26ab
SOO	38.05	0.56 ± 0.05abc	0.68 ± 0.12abc	0.60 ± 0.13abc	0.48 ± 0.15ab	0.47 ± 0.05a	0.70 ± 0.13c	0.68 ± 0.07a	0.78 ± 0.04a	0.67 ± 0.45a	0.77 ± 0.06a	0.67 ± 0.15a
SPO	39.56	0.33 ± 0.02a	0.37 ± 0.11a	0.35 ± 0.12a	0.25 ± 0.08a	0.24 ± 0.04a	0.36 ± 0.05a	0.43 ± 0.03a	0.42 ± 0.05a	0.37 ± 0.24a	0.49 ± 0.07a	0.38 ± 0.13a

[a] Retention time (min)

[b] STO and SML are soybean oil samples with 180 °C thermal oxidation and with methylene blue under light irradiation, respectively

[c] Mean ± standard deviation ($n = 3$)

[d] Different letters indicate significant differences within same row at $p < 0.05$

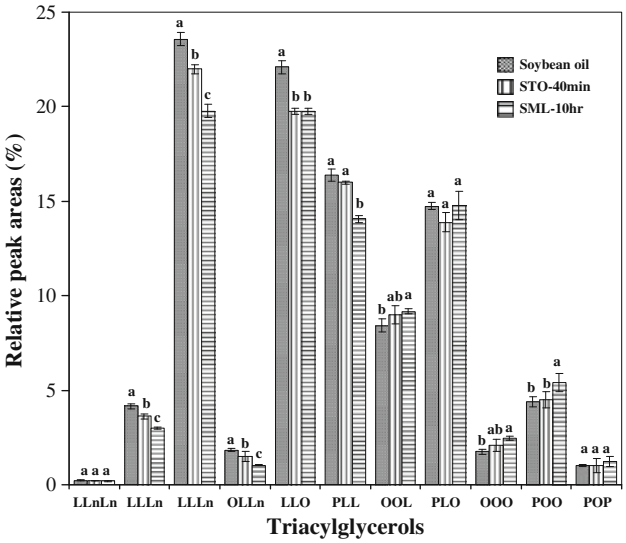

Fig. 3 Comparison of relative contents of major triacylglycerols in soybean oil with 40 min-thermal oxidation and 10 h-photosensitized oxidation

Comparison of TAG Between Thermally Oxidized and Photosensitized Soybean Oils

Comparison of relative peak areas of TAG in soybean oils with 40 min-thermal oxidation and 10 h-photosensitized oxidation are shown in Fig. 3. The 40 min-thermally oxidized and 10 h-photosensitized soybean oils showed a similar degree of oxidation based on the depletion of headspace oxygen (Fig. 1). Some TAG showed different distribution patterns between 40 min-thermally oxidized and 10 h-photosensitized soybean oils. Photosensitized soybean oil had significantly lower relative peak areas of TAG including LLLn, LLL, OLLn, and PLL and significantly higher relative peak areas of POO than thermally oxidized samples ($p < 0.05$). Relative contents of LLnLn, LLO, OOL, PLO, OOO, and dipalmitoyloleoylglycerol (POP) were not significant between thermally oxidized and photosensitized soybean oils ($p > 0.05$). The results of this study implied that TAG in soybean oil were influenced differently from photosensitized oxidation and thermal oxidation. Photosensitized oxidation accelerated the relative peak areas of TAG containing linoleic and linolenic acids faster than thermal oxidation.

The oxidative stability of TAG including LLLn, LLL, OLLn, and PLL was significantly lower in photosensitized oxidation than thermal oxidation, which may be due to the singlet oxygen oxidation. Methylene blue is a well-known type II sensitizer and can generate singlet oxygen upon visible light irradiation. Singlet oxygen oxidation may play important roles in the significantly lower stability of TAG containing linoleic and linolenic acids due to the fast reactions with polyunsaturated fatty acids [1]. Neff et al.

[23] determined the effects of TAG composition and structures on the oxidative stability of soybean oil oxidized at 60 °C. The researchers reported that the rate of peroxidation was positively correlated with the position of linoleic acid in the glycerol backbone.

ECN could be one of the indicators determining the oxidative stability of soybean oil during oxidation. Especially, TAG with below 44 ECN are more susceptible to oxidation than those above 46 ECN when TAG are made of 16 or 18 carbon numbered fatty acids. This trend was clearly observed in both methylene blue photosensitization and thermal oxidation in soybean oil. However, the distributions of some TAG did not show consistent patterns during oxidation. For example, the relative peak areas of PLO (46) in both thermally oxidized and photosensitized soybean oil decreased significantly and then started to increase (Table 2). More studies are needed to understand these inconsistent changes of TAG during oxidation.

PCA Approach of TAG in Thermally Oxidized and Photosensitized Soybean Oils

Score plots of the PCA for TAG profiles in soybean oil treated with thermal oxidation and photosensitization are shown in Fig. 4. First principal component (PC1) and second principal component (PC2) express 50.399 and 24.938% of the data variability, respectively. Thermally oxidized soybean oils from 10 to 60 min oxidation were clustered together and those with 1-10 h photosensitization were grouped together (Fig. 4). Thermally oxidized and photosensitized soybean oils can be distinguished clearly based on the PCA results on the TAG profiles.

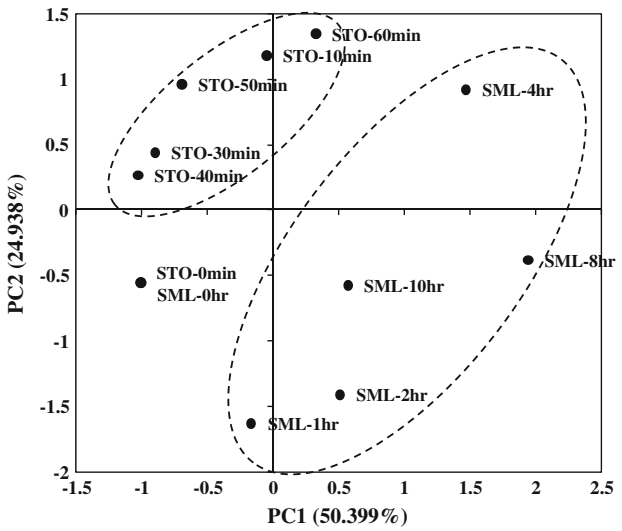

Fig. 4 A score plot of the PCA for triacylgylcerol distributions in thermally oxidized and photosensitized soybean oils

PCA approaches have been used to differentiate the 93 plant oils using the distribution of TAG analyzed by HPLC [24] and to detect adulterated olive oil with soybean oil through comparing TAG profiles [25]. TGA profiles could be useful parameters for differentiating the oxidation state of fats and oil treated with thermal oxidation and photosensitization as shown in this study.

Conclusion

Distribution of TAG and fatty acids in soybean oil treated with 180 °C thermal oxidation and methylene blue photosensitization were analyzed and compared. TAG with lower than 44 ECN including LLLn (40), LLL (42), OLLn (42), LLO (44), and PLL (44) decreased and those with higher than 46 ECN such as OOL (46) increased in relative peak areas during oxidation. TAG including LLLn, LLL, OLLn, and PLL in photosensitized soybean oil decreased significantly more than in thermally oxidized soybean oil, which implies singlet oxygen generated from photosensitization reacted quickly with the double bonds in TAG. TAG containing linoleic and linolenic acids are more susceptible to methylene blue photosensitization than thermal oxidation under the current experimental conditions. Photosensitized and thermal oxidized soybean oils were clearly differentiated based on PCA results for the TAG profiles.

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