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RESEARCH ARTICLE

Effects of Monoacylglycerols on the Oil Oxidation of Acidic Water/Perilla Oil Emulsion under Light in the Presence of Chlorophyll

Nakyung Kim and Eunok Choe

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Abstract Monoacylglycerol (MAG) effects on the oil photooxidation of an emulsion containing chlorophyll were studied. The emulsion consisted of equal weights of hexane-extracted perilla oil and 0.5% acetic acid, and 4 ppm chlorophyll b and MAG at 0, 1, or 1.5% were added. The oxidation was performed under 1,700 lx light at 25°C for 48 h. Singlet oxygen was involved in the oil oxidation of the emulsion containing chlorophyll under light. MAG protected chlorophyll and polyphenol compounds from degradation during the oxidation of the emulsion under light. MAG significantly decreased and decelerated headspace oxygen consumption and hydroperoxide production in the emulsion, and thus acted as antioxidant in photooxidation of the acidic water/perilla oil emulsion containing chlorophyll. Antioxidant activity of MAG in the photooxidation of the emulsion could be due to combined results of increased retention of polyphenols and decreased oxygen diffusion by forming a physical barrier.

Keywords: monoacylglycerol, acidic water/perilla oil emulsion, photooxidation, chlorophyll, polyphenol compound

Introduction

Monoacylglycerol (MAG) is naturally found in crude fats and oils since cellular lipases interact with water to hydrolyze glycerols or sterols (1). MAG content of soybean oil ranges from 0.07 to 0.11% (2), and olive and rapeseed oils contain MAG at <0.2% (1). MAG has hydrophilic 2 hydroxy groups and 1 hydrophobic hydrocarbon, and thus is surface active to be used as emulsifier; its hydrophilic-lipophilic balance (HLB) is in the range of 3.4 to 3.8 (1). It is positioned at the interface of the emulsion, forming a protective membrane that prevents the droplets from coming close together enough to aggregate. MAG is the most common emulsifier in food industry, which accounts for about 70% of emulsifier usage (3).

Light accelerates the oxidation of oil by providing the energy to abstract hydrogen from lipids, producing reactive lipid radicals. Shorter wavelengths of light showed more detrimental effects than longer wavelengths (4). It has been reported that the effect of light on the oil oxidation becomes less as the oxidation temperature increases (5). Light also increases the lipid oxidation in the presence of photosensitizer such as chlorophyll by producing singlet oxygen which is more reactive than atmospheric triplet oxygen (2).

MAG increased the autoxidation of soybean oil (6,7) by decreasing surface tension of oil and increasing the oxygen diffusion into the oil. Few studies have been reported on the MAG effects on the oxidation of emulsion as well as other edible fats and oils. There was no report on the MAG effects on the photooxidation of oil in the emulsion system in the presence of chlorophyll to our knowledge so far. Therefore, this study was performed to show the effects of MAG on the oil oxidation of the emulsion containing photosensitizer under light as a model system of colored salad dressing. Contents changes of polyphenol compounds and chlorophyll in the emulsion during photooxidation were also studied.

Materials and Methods

Materials and chemicals Perilla oil was extracted from raw perilla seeds by mixing with *n*-hexane at 40°C for 2 h,

Nakyung Kim, Eunok Choe (✉)
Department of Food and Nutrition, Inha University, Incheon 402-751, Korea
Tel: +82-32-860-8125; Fax: +82-32-873-8125
E-mail: eochoe@inha.ac.kr

filtering, and then desolventization at 40°C. The hexane-extracted perilla oil (PO) was passed through a column packed with alumina to remove tocopherols (8). *n*-Hexane in the eluent was completely removed by a rotary evaporator (N,N series; Eyela, Tokyo, Japan) at 40°C to have tocopherol-stripped perilla oil (TSPO). MAG (Excel O-95R) whose MAG content was 81.7% was obtained from Namyung Commercial Co., Ltd. (Seoul, Korea) and it additionally contained phosphoric acid (0.05%), diaclyglycerols (DAG, 16.0%), and free fatty acids (FFA, 2.3%). Chlorophyll b, diazabicyclooctane (DABCO), xanthan gum, cumene hydroperoxide (CuOOH), alumina (Type WN-3), BF_3 in methanol, caffeic acid, Folin-Ciocalteau reagent, lipid standards (monolinolein, monoolein, dilinolein, diolein, trilinolein, trilolein, and linoleic and oleic acids), standard fatty acid (palmitic, stearic, oleic, linoleic, and linolenic acids) methyl esters, and α-, β-, γ-, and δ-tocopherols were products of Sigma-Aldrich (St. Louis, MO, USA). Pheophytin b was prepared by adding 0.01 M HCl solution to standard chlorophyll b according to the method of Redfearn and Friend (9). *n*-Hexane, methanol, ethylacetate, water, and isopropanol in HPLC grade were purchased from J.T. Baker (Philipsburg, NJ, USA). All other chemicals were of analytical grade.

Preparation of an emulsion and its photooxidation An emulsion of this study, designated as acidic water in tocopherol-stripped perilla oil (AW/TSPO), basically consisted of 5% acetic acid (50 g), TSPO (50 g), xanthan gum (0.16 g), and chlorophyll b (0.4 mg). MAG was also added to the emulsion at 1 or 1.5% which is in the range for a common use as an emulsifier in food industry. Control sample was the emulsion which was not added with MAG, but with the same composition of other ingredients. All ingredients were mixed for 30 s and homogenized in an Ultra-Turrax T25 homogenizer equipped with an S25N-25F dispersing tool (IKA Instruments, Staufen, Germany) at 10,000 rpm for 6 min. The pH of the control emulsion, and emulsions added with MAG at 1 and 1.5% were 3.31, 3.04, and 2.85, respectively. Light was excluded during sample preparation as much as possible.

Four g of sample emulsions were transferred into 20-mL glass serum vials, which were then capped air-tight with rubber septa and aluminum caps. The vials were placed in an incubator (LBI-250; Daihan Labtech Co., Seoul, Korea) with fluorescent lights of 1,700 lx at 25°C for 48 h for oxidation. All samples were prepared in duplicate.

Determination of characteristics of oils Lipid composition of oils was determined by TLC and densitometry (10). The oils were loaded onto pre-coated Kieselgel 60 F_{254} TLC plates (Merck, Darmstadt, Germany) and developed in a mixture of hexane, diethyl ether, and acetic acid (50:50:1, v/v/v). Each spot in the plates was identified with standard lipids after coloring with iodine vapor and 5% sulfuric acid, and then heating at 200°C for 1 min. Content of each lipid class was quantified with an imaging densitometer (model GS-700; Bio-Rad, Hercules, CA, USA).

Fatty acid compositions of oils were analyzed by GC after esterification with 14% BF_3 in methanol (11). A gas chromatograph was a Younglin M600D GC (Younglin Co., Ltd., Anyang, Korea) equipped with a Supelcowax capillary column (30 m×0.53 mm, 1.0 μm thick; Supelco Inc., Bellefonte, PA, USA) and a flame ionization detector. Temperatures of the oven, the injector, and the detector were 200, 270, and 280°C, respectively. The nitrogen flow rate was 5 mL/min, and the split ratio was 33:1. Each fatty acid in the chromatogram was identified by comparing the retention times with those of standard fatty acid methyl esters, and quantified by the peak areas. Contents of peroxides in oils were determined by AOCS Cd 8-83 method (12).

Contents of tocopherols in oils were determined by HPLC (13). The oil was dissolved in 5 mL *n*-hexane and filtered through a 0.2-μm PTFE membrane (Millipore, Molsheim, France). The filtrate (20 μL) was injected into a Younglin YL9100 HPLC (Younglin Co., Ltd.). A μ-porasil column (330 mm×3.9 mm, 10 μm size; Waters Co., Milford, MA, USA) and a fluorescence detector with an excitation wavelength of 290 nm and emission of 330 nm were used. The mobile phase was 0.2%(v/v) isopropanol in *n*-hexane with a flow rate of 2.0 mL/min. Each tocopherol was identified by comparing retention times with those of standard tocopherols, and quantified by using respective calibration curves.

Chlorophylls were determined by HPLC (14). The oil dissolved in methylene chloride (20 μL) was injected into a Younglin SP 930D HPLC (Younglin Co., Ltd.) equipped with a symmetry C18 column (5.0 μm, 4.6 mm i.d.×150 mm; Waters) and UV-Vis detector (ACME 930; Younglin Co., Ltd.) set at 438 nm. The eluting solvent was a mixture of ethylacetate, methanol, and water (50:37.5:12.5, v/v/v) at a flow rate of 1 mL/min. Quantification as well as identification were performed with standard chlorophylls.

Polyphenols in oils were determined by spectrophotometry using a Folin-Ciocalteau reagent (15). The oil (3 g) was dissolved in 10 mL *n*-hexane, and the solution was extracted with 2 mL of methanol-water mixture (60:40, v/v) 3 times. Combined extracts were evaporated at 40°C, and the residue was redissolved in 1 mL methanol. The aliquot was diluted with distilled water and then 0.3 mL Folin-Ciocalteau reagent was added. After 3 min, 0.5 mL saturated Na_2CO_3 solution was added. The solution was diluted to 5 mL with distilled water. The absorbance of the solution was measured at 725 nm after 1 h standing at room temperature. Contents of polyphenols in oils were expressed as caffeic acid.

Evaluation of oil oxidation in the emulsion Oil oxidation in the emulsion was determined by headspace oxygen consumption and hydroperoxides production. Headspace oxygen consumption was determined by subtracting headspace oxygen content of samples at time t from the headspace oxygen content of samples at time 0. Decrease in oxygen content in the headspace of a gas-tight sample bottle was assumed to be oxygen consumption by oil in the emulsion due to its oxidation, as shown in previous researches (14,16). Headspace oxygen content was determined by GC (17). The headspace gas (0.5 mL) of the sample vial was injected into an YL 6100 GC with YL6100 autosampler (Younglin Co., Ltd.). The detector was a thermal conductivity detector and the column was a stainless steel column packed with 80/100 mesh molecular sieve 13X (1.83 m× 0.32 cm; Alltech, Deerfield, IL, USA). Helium (99.995%) was a carrier and auxiliary gas. Temperatures of the oven, the injector, and the detector were 35, 100, and 140°C, respectively. Areas of the oxygen peak in the chromatograms were converted to μmol of oxygen in 1 mL of headspace gas under the assumption that atmospheric air contains 20.946% oxygen, which gives the result that 1 mL of air is equivalent to 9.35 μmol oxygen (14).

Hydroperoxide content in the emulsion was determined by the ferric thiocyanate method (17). The emulsion (0.3 mL) was mixed with a 1.5 mL mixture of isooctane and 2-propanol (3:1, v/v) for 10 s followed by centrifugation (Mikro 200; Hettich, Tuttlingen, Germany) at 1,000×*g* for 2 min to take an organic layer, with 3 repetitions. Two-hundred μL of the combined organic layer was taken and a solution (2.8 mL) of methanol and chloroform (2:1, v/v), 3.94 M ammonium thiocyanate solution (15 μL), and 15 μL of 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$ solution were added in a respective order. After 20 min of standing at room temperature, the absorbance of the solution was read at 510 nm with a UV-Visible spectrophotometer (HP8453; Hewlett Packard, Wilmington, DE, USA). Hydroperoxide content of the oil was expressed with CuOOH in mmol/kg.

Data analyses SAS (Version 8.2; SAS Inst. Inc., Cary, NC, USA) and Microsoft Excel 2003 (Microsoft Corporation, Seoul, Korea) were used for statistical treatment of the data. Statistical treatment included Duncan's multiple range test at 5% significance level as well as determination of means and standard deviations (SD).

Results and Discussion

Characteristics of oils Characteristics of PO and TSPO are shown in Table 1. PO consisted of mainly triacylglycerols (TAG, 88.0%) with 1,2- DAG (6.1%), 1,3-DAG (1.3%), and FFA (4.7%). There was no MAG detected in PO. The only lipid class detected in TSPO was TAG. Fatty acid composition of TSPO was very similar to that of PO; the most abundant fatty acid was linolenic acid (59.0%), followed by oleic (18.3%) and linoleic (14.1%) acids. Palmitic (about 6%) and stearic (about 2%) acids were also present in both oils. PO contained peroxides at 0.10 meq/kg, but alumina column chromatography decreased peroxides contents in TSPO to 0.04 meq/kg. PO contained tocopherols totally at 397.7 mg/kg, with α-, γ-, and δ-tocopherol at 36.6, 342.3, and 18.9 mg/kg, respectively. However, there were no tocopherols detected in TSPO indicating all tocopherols in PO were removed by alumina column chromatography. Concentrations of polyphenols in PO and TSPO were 4.67 and 3.05 mg/kg, respectively. Chlorophyll was not detected in both oils.

Table 1. Characteristics of perilla oil (PO) and tocopherol-stripped perilla oil (TSPO)

	PO	TSPO
Lipid composition, %		
Monoacylglycerols	ND[1)]	ND
1,2-Diacylglycerols	6.1±0.18	ND
1,3-Diacylglycerols	1.3±0.12	ND
Free fatty acids	4.7±0.01	ND
Triacylglycerols	88.0±0.29	100
Fatty acid compositions, mg/100 g of oil (relative %)		
$C_{16:0}$	50.9±1.2 (6.1%)	47.3±0.98 (6.3%)
$C_{18:0}$	18.1±0.1 (2.2%)	17.9±0.29 (2.4%)
$C_{18:1}$	143.6±3.2 (17.3%)	136.5±1.69 (18.3%)
$C_{18:2}$	115.1±1.9 (13.9%)	104.9±1.62 (14.1%)
$C_{18:3}$	500.7±5.7 (60.4%)	440.1±7.39 (59.0%)
$C_{20:1}$	ND	ND
Peroxide value, meq/kg	0.10±0.04	0.04±0.03
Tocopherol contents, mg/kg		
α-	36.6±0.0	ND
γ-	342.3±12.7	ND
δ-	18.9±0.9	ND
Total	397.7±13.6	ND
Polyphenols contents, mg/kg	4.67±0.4	3.05±0.32
Chlorophylls contents, mg/kg	ND	ND

[1)]Not detected

MAG consisted of palmitic (5.6%), stearic (4.3%), oleic (70.4%), linoleic (13.4%), linolenic (4.0%), and eicosenoic (2.3%) acids. It contained α-, γ-, and δ-tocopherol at 28.9, 150.7, and 66.3 mg/kg, respectively. There were no polyphenol compounds, nor chlorophylls.

Effects of MAG on the fatty acid composition of the AW/TSPO emulsion containing chlorophyll under light The AW/TSPO emulsions were relatively stable throughout 48 h without phase separation. Fatty acid composition of AW/TSPO emulsions before photooxidation was similar to

Table 2. Effects of monoacylglycerol (MAG) on the fatty acid compositions of the acidic water/perilla oil emulsion containing chlorophyll b during oxidation under light at 25°C

MAG addition level (%)	Oxidation time (h)	Relative content (%)					U/S[2)]
		C16:0	C18:0	C18:1	C18:2	C18:3	
0	0	6.51±0.14[a1)]	2.46±0.14[a]	18.48±0.16[d]	14.08±0.57[a]	58.47±0.69[a]	10.15±0.35[ab]
	12	6.60±0.04[a]	2.43±0.04[a]	18.97±0.09[c]	13.81±0.02[a]	58.20±0.19[a]	10.08±0.10[ab]
	48	6.68±0.01[a]	2.48±0.02[a]	19.10±0.01[c]	13.81±0.10[a]	57.93±0.05[a]	9.91±0.04[b]
1	0	6.45±0.02[a]	2.40±0.00[a]	19.40±0.1[bc]	13.76±0.00[a]	57.99±0.13[a]	10.30±0.03[ab]
	12	6.36±0.06[a]	2.36±0.04[a]	19.33±0.21[bc]	13.76±0.02[a]	58.19±0.33[a]	10.47±0.13[a]
	48	6.65±0.05[a]	2.48±0.02[a]	20.01±0.34[a]	14.04±0.35[a]	56.82±0.76[a]	9.95±0.09[b]
1.5	0	6.39±0.11[a]	2.36±0.05[a]	19.64±0.14[ab]	13.78±0.03[a]	57.82±0.33[a]	10.42±0.21[a]
	12	6.40±0.08[a]	2.45±0.02[a]	19.75±0.02[ab]	13.87±0.11[a]	57.53±0.08[a]	10.31±0.07[ab]
	48	6.55±0.15[a]	2.40±0.03[a]	19.92±0.36[a]	13.84±0.13[a]	57.29±0.66[a]	10.18±0.23[ab]

[1)]Different superscripts mean significant differences at α=5% among samples within the same fatty acid.
[2)]Content ratio of unsaturated fatty acids to saturated fatty acids

that of TSPO, and was not significantly different among emulsions with different addition levels of MAG except oleic acid as shown in Table 2; relative content of oleic acid was significantly higher in the emulsion added with MAG (>19%) than in the control emulsion without MAG (18.5%) due to very high content (70.4%) of oleic acid in MAG. Fatty acid composition of the emulsions changed a little during photooxidation; there was a tendency that relative content of linolenic acid in the emulsion decreased during the oxidation under light. However, relative contents of oleic and palmitic acids increased, and the content ratios of unsaturated fatty acids to saturated fatty acids (U/S ratio) were decreased. This suggests an occurrence of oil oxidation in AW/TSPO emulsion containing chlorophyll under light. As the oil is oxidized, relative contents of more unsaturated fatty acids become decreased faster than those of less unsaturated fatty acids, resulting in decrease in U/S ratio (18).

Effects of MAG on the oil oxidation of the AW/TSPO emulsion containing chlorophyll under light Content of hydroperoxides in the control emulsion containing chlorophyll b was 2.77 mmol/kg before photooxidation, and increased to 59.02 mmol/kg after 48 h oxidation under light, due to the photooxidation of oil. However, when DABCO was added, the hydroperoxides content of the control emulsion after 48 h photooxidation was 42.20 mmol/kg, which was significantly lower than that without DABCO. DABCO is a well-known singlet oxygen quencher (14), and thus decreased hydroperoxide production in the emulsion added with DABCO implied singlet oxygen production and its involvement in the oil oxidation of the emulsion containing chlorophyll under light.

MAG effects on the oil oxidation evaluated by headspace oxygen consumption and hydroperoxide production in the AW/TSPO emulsion containing chlorophyll during photooxidation are shown in Fig. 1. Headspace oxygen consumption and hydroperoxide production increased with oxidation time in all samples due to the oil oxidation in the emulsion. The oil reacts with oxygen to produce primary oxidation products, hydroperoxides, thus resulting in increased oxygen consumption and hydroperoxide production. Headspace oxygen consumption and hydroperoxides contents in the emulsion added with MAG were significantly lower (p<0.05) than that in the control emulsion which was not added with MAG during oxidation under light. There was a tendency that AW/TSPO emulsion with more MAG showed less headspace oxygen consumption and hydroperoxide production. This indicates that addition of MAG decreased headspace oxygen consumption and hydroperoxide production in the oil during photooxidation of the emulsion containing chlorophyll.

Headspace oxygen consumption and hydroperoxide production in the AW/TSPO emulsions containing chlorophyll under light for 48 h were correlated with oxidation time very well (r^2>0.93) as shown in Table 3. Rate of headspace oxygen consumption, which is a slope of the regression line ('a' value) between oxygen consumption and oxidation time, was the highest in the control emulsion (0.077 μmol O_2/mL/h, r^2=0.989), and the lowest in the emulsion added with MAG at 1.5% (0.032 μmol O_2/mL/h, r^2=0.978). This indicates that addition of MAG slowed down the oxygen consumption in the headspace of the emulsion containing chlorophyll under light. Rate of hydroperoxides content increase was lower in the AW/TSPO emulsion added with MAG; rates of hydroperoxides content increase in the control emulsion and the emulsion added with MAG at 1 and 1.5% were 1.097, 0.341, 0.293 mmol/kg/h, respectively. Thus, MAG decelerated hydroperoxide production in the AW/TSPO emulsion in the presence of chlorophyll during photooxidation.

The results on headspace oxygen consumption and

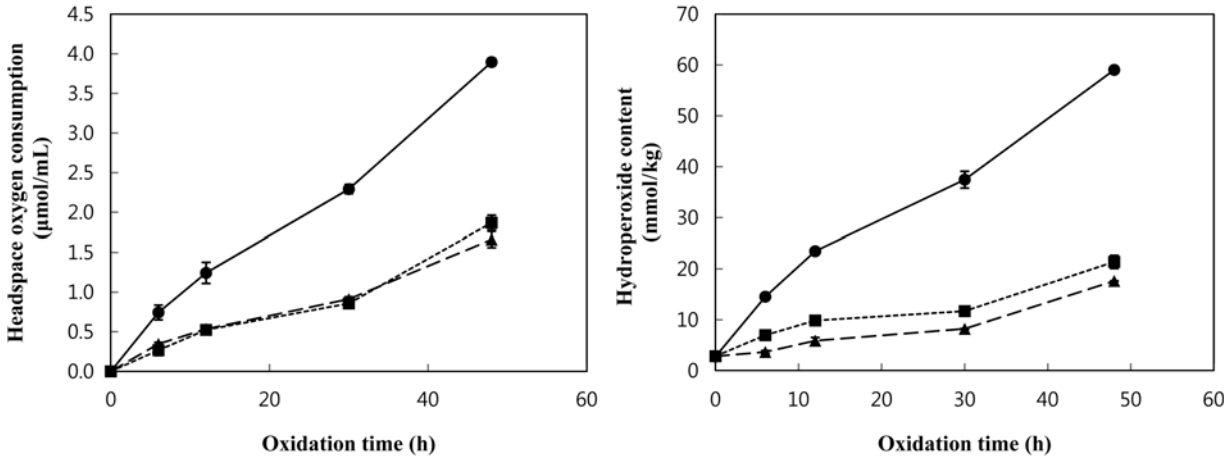

Fig. 1. Effects of monoacylglycerol (MAG) on the headspace oxygen consumption and hydroperoxide contents in acidic water/perilla oil emulsion containing chlorophyll b during oxidation at 25°C under light. MAG concentration ● 0%, ■ 1%, ▲ 1.5%

Table 3. Effects of monoacylglycerol (MAG) on the regression analysis between headspace oxygen consumption/hydroperoxide contents and oxidation time during photooxidation of acidic water/perilla oil emulsion at 25°C

	MAG level added (%)	Regression analysis[1)]		
		a	b	r^2
Headspace oxygen consumption	0	0.077	0.162	0.989
	1	0.036	0.008	0.962
	1.5	0.032	0.076	0.978
Hydroperoxide contents	0	1.097	6.385	0.982
	1	0.341	4.019	0.933
	1.5	0.293	2.008	0.930

[1)]Headspace oxygen consumption (μmol/mL)/hydroperoxide contents (mmol/kg)=a×oxidation time (h)+b, r^2=determination coefficient

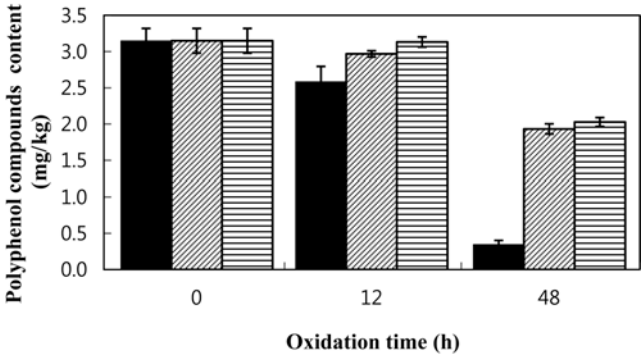

Fig. 2. Effects of monoacylglycerol (MAG) on the polyphenol compounds contents in acidic water/perilla oil emulsion containing chlorophyll b during oxidation at 25°C under light. MAG concentration ■ 0%, ▨ 1%, ▤ 1.5%

hydroperoxides production in the AW/TSPO emulsions containing chlorophyll clearly indicate that addition of MAG decreased and decelerated the oxidation of oil in the emulsion in the presence of chlorophyll under light; thus MAG acted as antioxidants. This is noteworthy since MAG acted as prooxidant in the autoxidation of soybean oil (7). Lower and slower increase in headspace oxygen consumption and hydroperoxides production in the emulsion added with MAG compared to the control emulsion could be due to the effects of MAG on the emulsion structure, fatty acid composition change, and the pH which might affect singlet oxygen production. At the concentration of about 1% of an emulsifier, the packing of surfactant molecules such as MAG at the oil and water interface becomes tighter; hence the membrane acts as an efficient barrier to the diffusion of lipid oxidation initiators into the oil droplets (19), which results in decreased oil oxidation in the emulsion. Secondly, the emulsion added with MAG contained more oleic acid and less linoleic and linolenic acids, and thus could be oxidized slower than the control emulsion without MAG. The oxidation rate of fatty acids becomes lower as their unsaturation decreases (18). Finally, MAG could have affected singlet oxygen production in the emulsion by lowering the pH since MAG used in this experiment contained phosphoric acid. The pH of the emulsion added with MAG at 0, 1, or 1.5% was 3.31, 3.04, or 2.85, respectively. Singlet oxygen is produced at lower rate in more acidic conditions (20-22), and thus less amount of highly reactive singlet oxygen was produced in the emulsion added with MAG, resulting in less and slower oxidation than the control emulsion without MAG.

Changes in naturally present polyphenol compounds in perilla oil and added chlorophyll during photooxidation of the AW/TSPO emulsion Figure 2 shows content changes of naturally present polyphenol compounds during oxidation of chlorophyll-containing AW/TSPO emulsions under light. Initial content of polyphenol compounds in the emulsion containing chlorophyll was 3.15 mg/kg before

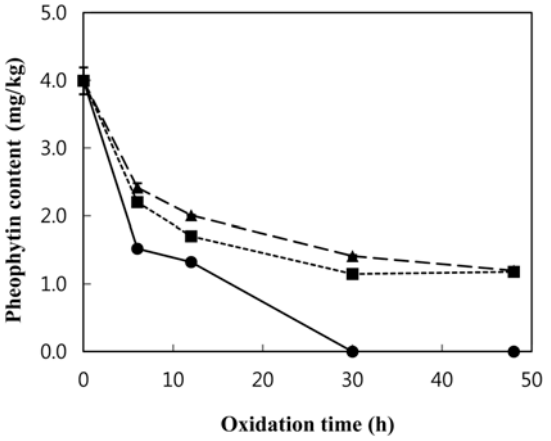

Fig. 3. Effects of monoacylglycerol (MAG) on the pheophytin contents in acidic water/perilla oil emulsion during oxidation at 25°C under light. MAG concentration ● 0%, ■ 1%, ▲ 1.5%

oxidation and decreased during oxidation under light, indicating their degradation. The emulsion added with MAG showed lower degradation of polyphenol compounds; 28.3 and 89.1% of polyphenol compounds were degraded in the control emulsion after 12 and 48 h oxidation, respectively. However, 6.2 and 38.9% of polyphenol compounds were degraded in the emulsion added with MAG at 1% after 12 and 48 h oxidation, respectively, and the emulsion added with MAG at 1.5% showed 1.0 and 35.9% degradation degree of polyphenol compounds after 12 and 48 h oxidation, respectively. This indicates that MAG protected polyphenol compounds from degradation during photooxidation of AW/TSPO emulsion containing chlorophyll. Polyphenol compounds are degraded when they exert an antioxidant activity, and oxygen and light can also oxidize polyphenol compounds (23-25). Tight packing structure of the emulsion by the addition of MAG could decrease oxygen diffusion and filter out some of the light, resulting in higher retention of polyphenol compounds in the AW/TSPO emulsion added with MAG. Higher retention of polyphenol compounds, antioxidants, in the AW/TSPO emulsion added with MAG than in the control emulsion could have contributed to high oxidative stability of the emulsion.

Chlorophyll b which was added to the emulsion was instantly converted to pheophytin b in the acidic condition. Content of pheophytin was continuously decreased from 3.99 mg/kg during oxidation of AW/TSPO emulsion under light as shown in Fig. 3, which indicates its degradation. Residual pheophytin content during photooxidation was higher in the AW/TSPO emulsion added with MAG than in the control emulsion. All pheophytins were disappeared in the control emulsion after 30 h, however, the emulsions added with MAG showed approximately 30% of pheophytin retention after 48 h. Degradation rate of pheophytin was

Table 4. Regression analysis between pheophytin contents and oxidation time in acidic water/perilla oil emulsion during oxidation under light at 25°C

	MAG level added (%)	Regression analysis[1)]		
		a	b	r^2
Pheophytin concentration	0	-0.0400	0.5415	0.843
	1	-0.0098	0.4487	0.734
	1.5	-0.0097	0.4888	0.852

[1)]Pheophytin concentrations (mg/kg)=a×oxidation time (h)+b, r^2= determination coefficient

higher in the control emulsion which did not contain MAG (Table 4); addition of MAG at 1 or 1.5% to the emulsion decreased the pheophytin degradation rate to 0.0098 and 0.0097 mg/kg/h, respectively, from 0.0400 mg/kg/h in the control emulsion. These results indicate that MAG decreased and decelerated pheophytin degradation during photooxidation of the AW/TSPO emulsion. Chlorophylls are degraded by light and both triplet and singlet oxygen (26,27), but MAG could protect pheophytin from degradation because MAG could decrease oxygen diffusion, filter out some of the light, and lower the pH to produce less singlet oxygen. Chlorophylls including pheophytin act as photosensitizer to produce singlet oxygen from triplet oxygen by energy transfer, and accelerate the oil oxidation (2). Thus, higher retention of pheophytin by MAG addition to the AW/TSPO emulsion could have caused higher oil oxidation, but our results were not; the emulsions with higher chlorophyll retention showed low and slow oil oxidation. This might suggest a possibility that MAG acted as a physical barrier for oxygen and light to increase the oil oxidation rather than via chemical mechanism such as oxygen and/or lipid radical quenching.

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