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Effects of conjugated linoleic acid on the degradation and oxidation stability of model lipids during heating and illumination

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Abstract

The effects of methyl conjugated linoleate (MCLA) on the degradation and oxidative stability of model lipids, methyl linoleate (ML), methyl oleate (MO) and methyl stearate (MS), during heating at 100, 150 or 200°C for 3 h or illumination at 4000 lux for 14 days were studied. Results showed that under either temperature treatment or illumination, MCLA was the most susceptible to degradation, followed by ML, MO and MS. At 100°C, peroxide formation was the main reaction in each model lipid. However, at 150°C, peroxide formation was the main reaction in the initial period of heating, followed by degradation. At 200°C, the degradation was the major reaction. In contrast, peroxide formation was the main reaction for each model lipid during illumination. The addition of MCLA may slow degradation of each model lipid during heating. However, the oxidation stability of the whole system (model lipid plus MCLA) may also be decreased. Under light storage, the effect of MCLA was insignificant. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Conjugated linoleic acid (CLA) belongs to a group of octadecadienoic acids containing two conjugated carbon–carbon double bonds. CLA is primarily present in meat and dairy products of ruminant origin (Ha, Grimm & Pariza, 1989; Chin, Liu, Storkson, Ha & Pariza, 1992; Werner, Luedecke & Shultz, 1992; Shantha, Ram, O'Leary, Hicks & Decker, 1995; Ip, Scimeca & Thompson, 1994; Shantha, Crum & Decker, 1994). In addition, CLA is also present in human blood, tissues and breast milk (Haumann, 1996). Some authors postulate that CLA may be formed in vivo from linoleic acid through free-radical-mediated oxidation, or through reaction between linoleic acid radical and protein (Cawood, Wickens, Iversen, Braganza & Dormandy, 1983; Dormandy & Wicken, 1987).

Several studies have suggested that CLA shows anti-atherosclerotic (Lee, Kritchevsky & Pariza, 1994) and anti-carcinogenic (Belury, Nickel, Bird & Wu 1996; Ha, Storkson & Pariza, 1990; Ip, Chin, Scimeca & Pariza, 1991; Ip & Scimeca, 1997) effects. However, the exact mechanism remains uncertain. If CLA can be proved to be an effective antioxidant, it would partially explain the anti-carcinogenic mechanism. In a study dealing with the inhibition of benzo[α]pyrene-induced mouse forestomach neoplasia, Ha et al. reported that CLA is an effective antioxidant, and the activity is higher than α-tocopherol. The oxidation modification of CLA, into a metal chelator that subsequently stops radical generation, has been suggested to explain the potent antioxidant activity of CLA (Ha et al.). In a later study, Ip et al. (1991) further demonstrated that CLA may reduce the formation of thiobarbituric acid reactive substance (TBARS) in mammary gland. In contrast, Van den Berg, Cook and Tribble (1995) found that CLA is not able to retard the oxidation of a model membrane 1-palmitoyl-2-linoleoyl phosphatidylcholine. Also, CLA was found to be more susceptible to oxidation than linoleic acid when incubated in air separately (Van den Berg et al.). Chen, Chan, Kwan and Zhang (1997) found that CLA is a pro-oxidant in canola oil heated at 90°C. As these results are controversial, the pro-oxidant or antioxidant effect of CLA in lipids needs to be further investigated. The objectives of this study were to (1) investigate the effect of CLA on the oxidation stability of model lipids and (2) determine the degradation of

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model lipids in the presence of CLA during heating and illumination.

2. Materials and methods

2.1. Materials

Three model lipid standards, methyl stearate (MS), methyl oleate (MO) and methyl linoleate (ML), and methyl conjugated linoleate (MCLA), as well as internal standard methyl heptadecanoate, were purchased from Nu-Chek-Prep Inc. (Elysian, MN, USA). Reagents, including concentrated hydrochloric acid, hydrogen peroxide, barium chloride, ferrous sulfate, ferric chloride and ammonium sulfocyanate were from Sigma Co. (St. Louis, MO, USA). Solvents, including *n*-hexane, methanol and chloroform were of analytical grade and were from Merck Co. (Darmstadt, Germany). *n*-Hexane used for GC was HPLC grade and from Mallinckrodt Co. (Kentucky, USA).

2.2. Instrumentation

The GC instrument consisted of a HP model 6890 gas chromatograph with a flame ionization detector (FID) and a split/splitless injector. (HP Co., Palo Alto, CA, USA). A HP-INNOWAX capillary column (30 m×0.32 mm i.d.) with a 0.25-μm film thickness was used. The DU-70 double beam spectrophotometer was from Beckman Co. (Fullerton, CA, USA). The Sorvall high speed centrifugator (model RC5C) was from Du Pont Co. (Willington, DE, USA). The light measurement meter (model TES-1330) was from Tai-Se Electronic Co. (Taipei, Taiwan).

2.3. Methods

2.3.1. Heating

2.3.1.1. Model lipids. Ten milligrams each of MS, MO and ML were placed, separately, in screw-capped vials. Vials were immersed in an oil bath (40×30×20 cm), containing about 12 l oil with a temperature preset at 100, 150 or 200°C, and the heating time was 180 min. These temperatures were chosen because several food items such as instant noodles are often fried at 150°C or donut-fried at 200°C. For each temperature treatment, 90 vials were used. Three vials were collected from each oil bath every 20 min and inserted into an ice box (2°C) to terminate the reaction. All the vials were stored in a freezer (−26°C) for subsequent analyses of peroxide value (POV) and the residual amounts of MS, MO and ML. Triplicate analyses were conducted for each temperature treatment and a total of 270 vials was used. Likewise, 30 vials, containing 10 mg MCLA each, were heated in an oil bath for each temperature and a total of 90 vials was used. Three vials were collected from the oil bath every 20 min for a total of 180 min, and the residual amounts of MCLA were determined.

2.3.1.2. Model lipids plus conjugated linoleic acid. Ten milligrams each of MO and ML were mixed with 10% MCLA (1.0 mg) separately in a 2-ml screw-capped vial. For pretreatment of MS, which is present in solid form, the mixture containing 2.0 g MS and 0.2 g of MCLA, was dissolved in hexane and diluted to volume (10 ml) in a tube. After mixing thoroughly, 50 μl MS containing 10.0 mg MS and 1.0 mg MCLA were collected and transferred to a 2-ml vial. The mixture was evaporated to dryness under nitrogen. Vials were sealed and heated in an oil bath containing about 12 l oil, with a temperature preset at 100, 150 or 200°C, and the heating time was 180 min. For each temperature treatment, 90 vials were used. Three vials were collected every 20 min and then inserted into an ice box (2°C) to terminate the reaction. All the vials were stored in a freezer (−26°C) for subsequent analyses of POV and the residual amounts of MS, MO, ML and MCLA. Triplicate analyses were conducted and a total of 270 vials was used.

2.3.2. Illumination

2.3.2.1. Model lipids. Ten milligrams each of MO and ML were placed separately in a 2-ml transparent vial. Two milligrams MS in solid form was pretreated by dissolving in hexane and diluted to volume (10 ml) in a tube. After mixing thoroughly, 50 μl containing 10.0 mg MS was collected and transferred to a 2-ml transparent vial. The mixture was evaporated to dryness under nitrogen so that a thin film was formed uniformly on the bottom surface of the vial. All the vials were illuminated under a fluorescent tube (40 cm long) in an incubator at 10°C for 2 weeks. The light intensity was 4000±100 lux and the distance between the fluorescent tube and vials was about 40 cm. Two vials were randomly collected every two days, wrapped in aluminium foil, and stored in a freezer (−26°C) for subsequent analyses of POV and residual amounts of each model lipid. Duplicate analyses were conducted and a total of 48 vials was used. Similarly, 16 vials containing 10 mg MCLA, each, were also tested. Two vials were collected every two days for a total of two weeks for determination of residual amount of MCLA.

2.3.2.2. Model lipids plus conjugated linoleic acid. Ten milligrams each of MO and ML were mixed with 10% MCLA (1.0 mg), separately, in a screw-capped vial. For pretreatment of MS in solid form, the mixture containing 2 g MS and 0.2 g MCLA was dissolved in hexane and diluted to volume (10 ml) in a tube. After thorough mixing, 50 μl containing 10 mg MS and 1 mg MCLA were collected and transferred to a 2-ml vial. The mixture was evaporated to dryness so that a thin film was

formed uniformly on the bottom surface of the vial. All the vials were illuminated in the same way as model lipids as described above. A total of 48 vials was used.

2.4. Analysis of MS, MO, ML and MCLA

Each treated sample was mixed with methanol/chloroform (5/3, v/v) and diluted to 1 ml in a vial, after which 20 μl were collected and evaporated to dryness under nitrogen. One millilitre hexane solution, containing 20 ppm internal-standard methyl heptadecanoate, was added to the sample, and 1 μl was collected and injected onto the GC for analysis of MS, MO, ML and MCLA. Helium was used as a carrier gas with a flow rate at 1.1 ml/min. The flow rates of hydrogen and air were 30 and 350 ml/min, respectively. A splitless mode was used for injection with a temperature of 200°C. Column temperature was programmed from 50°C, maintained for 1 min, then increased to 180°C at a rate of 20°C/min, and maintained for 30 min. The detection mode was flame ionization with a temperature at 240°C. The identification of MS, MO, ML and MCLA were carried out by comparison of unknown peaks with reference standards and cochromatography with added standards. For quantification, one concentration of internal standard methyl heptadecanoate (20 ng/μl) was mixed with six concentrations of MS (5–200 ppm), MO (5–200 ppm), ML (5–200 ppm) or MCLA (5–200 ppm), and injected onto the GC. The calibration curve of each model lipid was prepared by plotting concentration ratio against area ratio. The content of each model lipid was quantified using the following formula:

$$W_{(\mathrm{mg})} = [(A/RRF)/A_{\mathrm{I.S.}}] \times W_{\mathrm{I.S.(ng)}} \times 5 \times 10^{-2}$$

where W is amount (mg) of MS, MO, ML or MCLA; A is peak area of MS, MO, ML or MCLA; $A_{\mathrm{I.S.}}$ is peak area of internal standard; $W_{\mathrm{I.S.}}$ is amount of internal standard; and RRF (relative response factor): $\frac{A/W}{A_{\mathrm{I.S.}}/W_{\mathrm{I.S.}}}$.

The residual amount of each model lipid and MCLA was subjected to analysis of variance and Duncan's multiple range test using Statistical Analysis System Institute ([SAS], 1985).

2.5. Determination of peroxide value

A method described by Schmedes and Holmer (1989) was used to determine POV in each treated sample. This method is based on the oxidation of Fe(II) to Fe(III) by peroxides; Fe(III) forms a violet complex with thiocyanate and this complex is quantitated spectrophotometrically. Triplicate analyses were conducted for heated samples while duplicate analyses were conducted for illuminated samples. The data were subjected to analysis of variance and Duncan's multiple range test using SAS (1985).

3. Results and discussion

3.1. Separation of model lipids and MCLA

The simultaneous separation of MS, MO, ML and MCLA isomers has been difficult because 12 MCLA isomers have been reported to be theoretically possible. Of the various isomers, 9-*cis*, 11-*trans* MCLA was found to be the most abundant in foods. (Chin et al., 1992) Initially we compared the effects of column variety, temperature programming condition, injector and detector temperature, and gas flow rate on the separation efficiency of model lipids and MCLA. After various studies, the most appropriate conditions for separation of model lipid and MCLA isomers were as follows: the capillary column was HP-INNOWAX (30 m×0.32 mm I.D.), film thickness 0.25 mm; the injection mode was splitless with a temperature 200°C; the initial column temperature was 50°C, maintained for 1 min, then increased to 180°C with a rate of 20°C/min, and maintained for 30 min; the detector temperature was 240°C; the carrier gas (He) flow rate was 1.1 ml/min.

Fig. 1 shows the GC chromatogram of model lipid and MCLA isomers. According to the separation order, the first four peaks were identified as heptadecanoate (internal standard), MS, MO and ML, while the last two peaks were tentatively identified as 9-*cis*, 11-*trans*/9-*trans*, 11-*cis* MCLA and 10-*trans*, 12-*cis* MCLA. The tentative identification of MCLA isomers was based on the retention behaviour on the HPLC chromatogram as well as by comparison with that reported by Ha et al. (1989). The reference data of MCLA standard provided

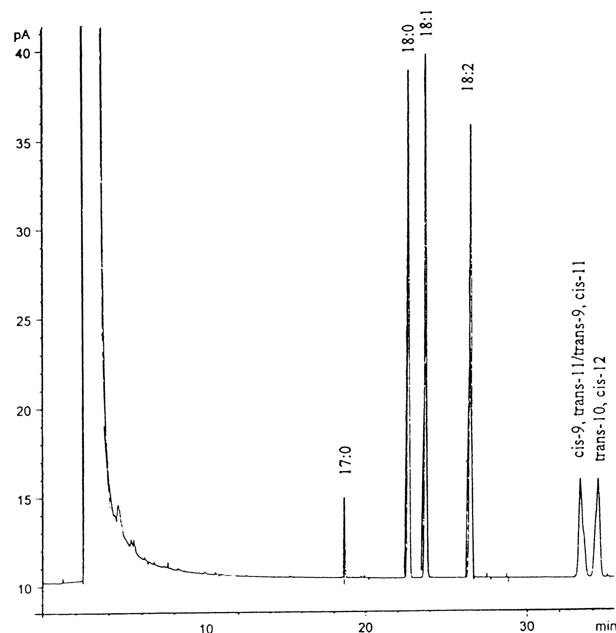

Fig. 1. GC chromatogram of model lipids and conjugated linoleic acid. Chromatographic conditions described in text.

by Nu-Check-Prep Co. also indicated that 9-*cis*, 11-*trans*/9-trans, 11-*cis* MCLA comprises about 41% while 10-*trans*, 12-*cis* MCLA comprised about 44%. The other MCLA isomers were not detected in this study, probably because they were present in trace amounts. The coefficients of variation for triplicate or duplicate analyses of model lipids and MCLA during heating or illumination ranged from 4.1 to 20.4% and 4.5 to 19.6%, respectively; the measurements of POV ranged from 3.2 to 21.2% and 1.2 to 17.4%, respectively.

3.2. Content changes of MS, MO, ML and MCLA during heating

Table 1 shows the residual amount (mg) of MS, MO, and ML in the presence of 10% of MCLA during heating at 100, 150 and 200°C for 3 h. When heated alone, the MS degradation was not pronounced until the temperature reached 200°C. When heated together with 10% MCLA at 100°C, the amount of MS only showed a minor change, indicating that no significant degradation of MS occurred. However, at 200°C, the addition of 10% MCLA showed a significant effect on MS stability in the initial period of heating. This is probably because MCLA was more susceptible to degradation than MS during heating. In addition, the MCLA itself was completely degraded after heating at 150 and 200°C for 60 and 20 min, respectively (Fig. 2). Thus, the effect of MCLA on the MS stability was minor after prolonged heating at 150 or 200°C. Also, MS was degraded to a greater extent at 200°C than 100 or 150°C.

Similar phenomena were found for the residual amount (mg) of MO in the presence of 10% MCLA during heating at 100, 150 and 200°C for 3 h. When heated together with 10% MCLA at 100°C, the MCLA had a significant effect on MO stability. As MCLA is theoretically more susceptible to formation of peroxides and free radicals than MO (Van den Berg et al., 1995), the oxidation stability of MO may be substantially decreased in the presence of 10% MCLA during heating. However, the oxidation stability of MO may also be increased in the event of complete degradation of MCLA at elevated temperature. It was found that, at 150 or 200°C, the MCLA (1 mg) was completely degraded after heating for 40 and 20 min, respectively, in the presence of MO (Fig. 2). Therefore, the addition of MCLA on the MO stability was insignificant after heating at high temperature for an extensive period of time. No significant degradation of MO occurred when heated at 100°C alone. On the contrary, at 150 and 200°C, the degradations of MO were more pronounced, and the residual amounts were less than 2 mg after heating for 140 and 40 min, respectively (Table 1). Also, MO was degraded at a faster rate without MCLA than with MCLA, which further demonstrated that the stability of MO was enhanced in the presence of MCLA at 100°C. This result implied that MCLA may act as an antioxidant during heating at low temperature. Likewise, at

Table 1
Residual amount (mg) of methyl stearate (MS), methyl oleate (MO) and methyl linoleate (ML) in the presence of 10% methyl conjugated linoleate during heating at 100, 150 and 200°C for 3 h

Model lipid	Temp.	Level of MCLA (%)	Time (min)[a]									
			0[b]	20	40	60	80	100	120	140	160	180
MS	100°C	0	10.0ab	8.5b	9.9ab	10.4a	10.2ab	10.5a	9.9ab	9.1ab	9.7ab	8.9ab
		10	10.0a	10.8a	10.8a	10.0a	10.8a	10.1a	10.3a	9.5a	9.8a	10.3a
	150°C	0	10.0a	9.7a	10.0a	10.3a	10.2a	9.6a	11.1a	10.4a	10.0a	10.0a
		10	10.0a	10.1a	9.5ab	9.8a	8.6bcd	9.4ab	9.1abc	8.3cd	8.0d	7.1e
	200°C	0	10.0a	7.1b	5.1c	4.1cd	4.4c	3.7cd	3.8cd	3.6cd	3.3d	3.6cd
		10	10.0a	9.1a	6.7b	5.6bc	5.5bc	4.5cd	4.3cd	4.4cd	3.4d	4.1cd
MO	100°C	0	10.0a	9.4a	9.1a	8.8a	9.2a	9.1a	8.0a	8.2a	8.6a	8.5a
		10	10.0a	9.9ab	10.3a	10.2a	10.2a	10.2a	9.2ab	9.3ab	9.1ab	8.7b
	150°C	0	10.0a	8.5b	6.4c	4.7d	3.9de	2.8ef	2.1fg	0.8gh	0.4h	0.4h
		10	10.0a	9.6a	7.8b	5.7c	4.9cd	4.1d	2.1e	2.0e	1.3e	1.3e
	200°C	0	10.0a	3.1b	1.4c	0.6cd	0.4d	0.5d	0.2d	0.3d	0.1d	0.0d
		10	10.0a	5.9b	2.4c	1.7cd	1.1cd	0.6d	0.7d	0.5d	0.7d	0.8d
ML	100°C	0	10.0a	10.2a	9.6ab	9.1ab	8.4bc	7.0cd	6.4d	5.0e	4.1ef	3.4f
		10	10.0a	10.1a	9.4a	9.8a	9.8a	9.8a	8.9a	7.5b	7.0b	4.3c
	150°C	0	10.0a	7.6b	4.5c	3.8cd	2.7d	1.0e	0.7e	0.7e	0.6e	0.3e
		10	10.0a	7.1b	4.6c	2.7d	2.5d	1.4e	0.4f	0.6f	0.4f	0.2f
	200°C	0	10.0a	4.3b	1.6c	0.6cd	0.4d	0.1d	0.1d	0.0d	0.0d	0.0d
		10	10.0a	4.8b	2.5c	1.3d	0.9cd	0.6def	0.2ef	0.0ef	0.0f	0.0f

[a] Average of triplicate analyses.
[b] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

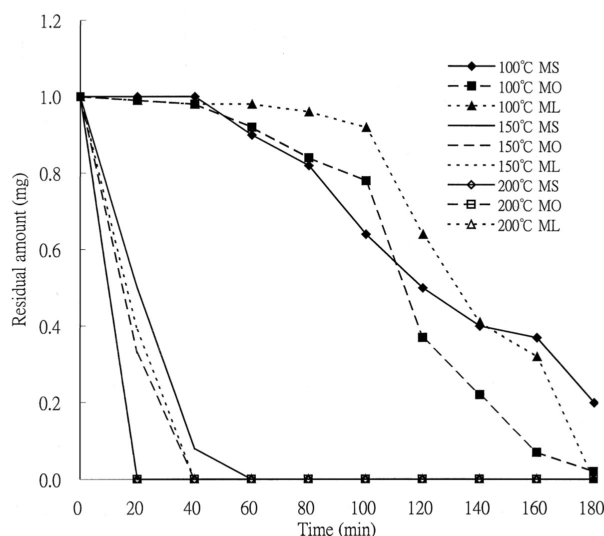

Fig. 2. Residual amounts (mg) of 10% MCLA (1 mg) in the presence of model lipids during heating.

high temperature (150 or 200°C), the addition of MCLA may provide some protective effect on MO degradation only during the initial period of heating time.

The residual amount (mg) of ML in the presence of 10% MCLA during heating at 100, 150 and 200°C for 3 h is also shown in Table 1. ML was the most susceptible to degradation at 200°C, followed by 150 and 100°C. With the exception of 150°C heating, the ML degradation was slower in the presence of 10% MCLA, implying that MCLA may exert a protective effect on ML at 100 or 200°C. Apparently this can be attributed to the unstable nature of MCLA. At 150 or 200°C, the MCLA (1 mg) was completely degraded after heating for 40 and 20 min, respectively, in the presence of ML (Fig. 2). In addition, the ML degradation proceeded faster with 10% MCLA at 200°C than at 100 or 150°C. As MCLA was more susceptible to degradation than ML, MCLA may provide a protective effect during heating. This result seemed to be contradictory to a report by Chen et al. (1997), who studied the effect of MCLA (0.1–1%) on the oxidation stability of canola oil heated at 90°C, and found that MCLA may have a pro-oxidant effect. This difference may be due to the temperature of heating and the concentrations of MCLA. Van den Berg (1994) further pointed out that the conjugated carbon–carbon double bond of MCLA is more prone to oxidative attack by free radicals than the methylene-interrupted double bond of ML. Interestingly, when 10 mg MCLA was heated alone at three temperatures, the degradation pattern was similar to that of 1 mg MCLA heated in the presence of model lipids. In comparison to model lipids, MCLA was the most susceptible to heating and illumination, followed by ML, MO and MS.

3.3. Changes of POV of MS, MO and ML in the presence of MCLA during heating

Table 2 shows the POV changes of MS, MO and ML in the presence of 10% MCLA during heating at 100, 150 or 200°C for 3 h. When heated alone at 150°C, the POV of MS rose with increasing heating time, implying that peroxide formation was the main reaction to proceed. This is probably because that MS may undergo dehydrogenation during heating, which in turn results in formation of hydroperoxides (Chang, Peterson & Ho, 1978). However, at 100°C, the POV of MS only showed a slight change. Interestingly, at 200°C, the POV rose in the first 60 min and then declined thereafter, which may be attributed to the degradation rate of peroxides being greater than the formation rate after prolonged heating. When heated together with 10% MCLA at 100 or 150°C, the POV changes showed a steady increase, probably because of the formation rate of peroxides being higher than the degradation rate. In addition, the MCLA may also be forming peroxides, thus contributing to the increase in POV. However, at 200°C, the POV increased greatly initially and then declined. Surprisingly, after prolonged heating for 140 min, the POV rose again. It has been reported that, after extensive heating treatment, the degradation products of peroxides may undergo subsequent oxidation to form peroxides (Frankel, Neff, Selke & Weisleder, 1982).

Similar trends were found for the POV changes of MO in the presence of 10% MCLA during heating at 100, 150 and 200°C for 3 h. When heated alone or together with 10% MCLA at 100°C, the POV of samples increased with increasing heating time, implying that peroxides were the main products formed during low-temperature heating. However, at 150 or 200°C, the POV rose in the initial period and thereafter declined. This result revealed that, after prolonged heating at a higher temperature, the degradation of peroxides may proceed faster than the formation. Also, the degradation of peroxides from MO was faster at 200°C than at 150°C. Interestingly, at 200°C, the products from breakdown of peroxides may again undergo oxidation to form peroxides after prolonged heating.

The POV change of ML in the presence of 10% MCLA during heating at 100, 150 and 200°C for 3 h is also shown in Table 2. When heated alone, the POV of ML followed an increasing order and reached a plateau after 100°C heating for 3 h, however, at 150 or 200°C, the POV reached a peak after 40 min heating and then declined. When heated together with 10% MCLA, the POV rose with increasing heating time at 100°C. However, at 150 or 200°C, an inconsistent change of POV occurred. This result indicated that peroxide formation was the main reaction to proceed in the initial period of heating, after which the peroxide degradation dominated, and again the oxidation of degradation products

Table 2
Peroxide value of methyl stearate (MS), methyl oleate (MO) and methyl linoleate (ML) in the presence of 10% methyl conjugated linoleate during heating at 100, 150 and 200°C for 3 h

Model lipid	Temp.	Level of MCLA (%)	Time (min)[a]									
			0[b]	20	40	60	80	100	120	140	160	180
MS	100°C	0	0.3a	0.4b	0.4c	0.5cd	0.7cd	0.6d	0.6d	0.6d	0.5d	0.5d
		10	0.6a	0.5a	8.9b	14.8c	26.9d	40.4e	63.7f	70.2g	91.8h	105i
	150°C	0	24a	28.1ab	54.7bc	73.7c	131d	171e	224f	237f	255g	311h
		10	1.5a	66.1b	117c	155c	220d	275e	332f	430g	465g	520h
	200°C	0	7.8a	219b	232bc	254c	242c	194bd	160d	155d	129e	66.2f
		10	4.0a	258b	215bc	198c	180d	172d	171d	202de	218ef	237f
MO	100°C	0	7.6a	12.1ab	20.2bc	27.5cd	30.1d	40.2e	64.4f	83.3g	107h	105h
		10	4.1a	9.1a	22.0b	44.9c	86.8d	154e	240f	317g	380h	502i
	150°C	0	1.5a	290b	460c	1126d	1085.0d	845e	750e	491f	399f	259g
		10	0.5a	248b	671c	837d	961e	746f	669cf	620f	584fg	523g
	200°C	0	0.1a	144b	83.8c	77.2cd	71.5d	47.3e	47.8e	62.2d	39.1f	24.5g
		10	18.5a	355b	227c	219c	158d	147de	130e	176f	258g	276g
ML	100°C	0	8.6a	23.2b	51.6b	103c	227cd	476d	890e	1244f	1893g	2028g
		10	1.6a	4.7a	8.4a	12.4a	157b	536c	1110d	1566e	1654f	1954g
	150°C	0	10.2a	218b	1265c	985d	641e	341f	173g	140gh	90.7hi	56.7i
		10	2.1a	854b	963c	721d	380e	306f	176g	242h	279hi	283i
	200°C	0	11.1a	152b	166b	155b	152b	155b	130c	102d	88.4de	80.5e
		10	12.9a	294b	118bc	84.4cd	60.5de	49.3e	95.7cd	76.5d	117ef	151f

[a] Average of triplicate analyses.
[b] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

occurred. The POV was found to be lower at 200°C than at 150°C, probably because of faster degradation of peroxides with high temperature treatment.

For model lipids MS and MO, heated at 100°C, the POV rose with increasing heating time in the presence of 10% MCLA. This may be explained by the enhanced oxidation stability of both MS and MO at low temperature (100°C), and thus the peroxide was formed mainly from MCLA. From this it is clear that MCLA was more susceptible to oxidation than ML, MO or MS (Table 1). For ML heated at 100°C, the addition of 10% MCLA did not show a significant effect on peroxide formation. It was also found that, at 200°C, the POV of MO or ML was lower than at 150°C. Apparently at a higher temperature, both the formation and degradation of peroxides may proceed simultaneously, and the degradation was assumed to be faster than the formation. In addition, when heated alone, the POV of each model lipid reached a maximum within a short period of time at 200°C, after which the degradation proceeded. However, when heated together with 10% MCLA at 200°C, the POV of each model lipid reached a peak shortly, after which the degradation proceeded, and again the peroxides ware formed. Although the 10% MCLA (1 mg) was completely degraded after 200°C heating for 20 min, it might also contribute to the increase of POV after heating for an extensive period of time.

By postulating a relationship between degradation of model lipids in the presence of MCLA and formation of peroxides in a model system containing both model lipids and MCLA during heating, it was assessed that MCLA mainly contributed to the formation of peroxides at 100°C. In addition, the presence of high residual amounts of each model lipid during heating was due to fast degradation of MCLA. However, after complete degradation of MCLA in the presence of model lipids at 150 or 200°C, the peroxides were mainly formed from model lipids. Furthermore, the degradation of model lipids and formation of peroxides were found to proceed simultaneously and faster with both increasing temperature and heating time.

The results shown above may be summarized as follows: (1) at 100°C, the peroxide formation is the main reaction to proceed, and MCLA contributes mainly to the formation of peroxide; (2) at 150°C, the peroxide formation dominates the initial period of heating for MO and ML, and then the degradation takes place (3) at 200°C, the peroxide degradation is the major reaction, and the effects of MCLA on stability of model lipids are minor because of complete destruction of 10% MCLA after heating for 20 min.

3.4. Content changes of MS, MO, ML and MCLA during illumination

Table 3 shows the residual amount (mg) of model lipids in the presence of 10% MCLA during illumination. When illuminated together with 10% MCLA, MS

Table 3
Residual amount (mg) of methyl stearate (MS), methyl oleate (MO) and methyl linoleate (ML) in the presence of 10% methyl conjugated linoleate during illumination for 14 days

Model lipid	Level of MCLA (%)	Time (day)[a]							
		0[b]	2	4	6	8	10	12	14
MS	0	10.0a	9.0ab	9.6ab	8.7b	9.1ab	8.8b	9.4ab	9.4ab
	10	10.0ab	10.0ab	9.9ab	10.4a	10.3a	9.9ab	9.9ab	9.7b
MO	0	10.0ab	10.2ab	9.7b	10.7a	10.2ab	10.2ab	9.9b	9.5b
	10	10.0a	9.7a	10.0a	10.3a	10.2a	9.4a	9.6a	9.7a
ML	0	10.0a	10.2a	10.2a	9.6a	7.4b	5.6c	4.0c	1.1d
	10	10.0a	9.9a	9.4a	8.0b	6.0c	3.4d	1.6e	0.7f

[a] Average of duplicate analyses.
[b] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

and MO showed substantial oxidation stability because the residual amount of both showed only minor change. In contrast, the residual amount of ML decreased with increasing illumination time. This result implied that the presence of MCLA might facilitate the degradation of ML under light storage, probably because of formation of peroxides from the former. When illuminated alone, ML was degraded faster than MS or MO, which can be attributed to the unstable nature of ML. Also, MCLA was the most susceptible to degradation during illumination, followed by ML, MO and MS (Table 3 and Fig. 3).

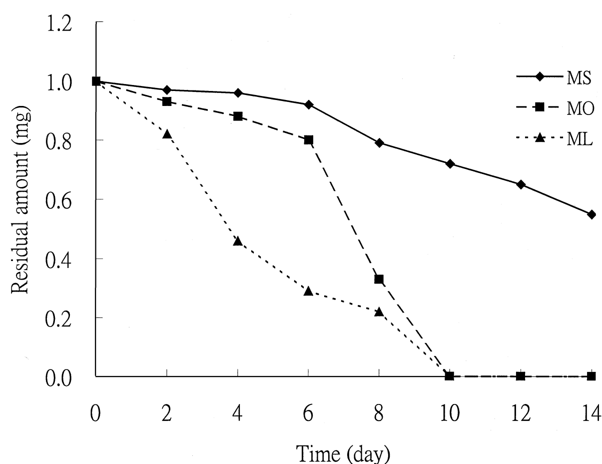

Fig. 3. Residual amounts (mg) of 10% MCLA (1 mg) in the presence of model lipids during illumination.

3.5. Changes of POV of MS, MO and ML in the presence of MCLA during illumination

Table 4 shows the POV of model lipids in the presence of 10% MCLA during illumination for 14 days. When illuminated alone, the POV of MS, MO or ML increased with increasing illumination time, showing that the peroxide formation was the main reaction to proceed. Also, ML was the most susceptible to peroxide formation, followed by MO and MS. When illuminated together with 10% MCLA, the POV of MS, MO or ML rose greatly, which can be attributed to formation of peroxides, from both MCLA and each model lipid. Apparently the formation of peroxides was found to be faster than the degradation when each of the model lipids and MCLA were illuminated together for 14 days.

In conclusion, MCLA may enhance the stability of each model lipid during heating at 100°C. The peroxide

Table 4
Peroxide value of methyl stearate (MS), methyl oleate (MO) and methyl linoleate (ML) in the presence of 10% methyl conjugated linoleate during illumination for 14 days

Model lipid	Level of MCLA (%)	Time (day)[a]							
		0[b]	2	4	6	8	10	12	14
MS	0	1.6a	8.4b	13.6cd	15.5e	14.8de	12.8c	12.9c	9.8bc
	10	0.5a	18.2b	39.5c	57.9d	87.8e	106f	103f	106f
MO	0	7.0a	12.2b	17.7c	26.1d	29.9e	39.9f	53.6g	64.7h
	10	8.8a	19.5ab	38.1b	69.0c	94.6d	135e	176f	225g
ML	0	4.2a	9.9a	34.8b	815c	1647d	2947e	4121f	4105f
	10	7.4a	13.9a	109b	1293c	2809d	3975e	4276e	3899e

[a] Average of duplicate analyses.
[b] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

formation is the main reaction to proceed for each model lipid during low-temperature heating (100°C) or illumination, while the peroxide degradation is the main reaction during high-temperature heating (200°C). MCLA is the most susceptible to degradation during heating or illumination, followed by ML, MO or MS. Also, the addition of 10% MCLA does not show a significant effect on oxidation stability of each model lipid at 200°C.

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