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ORIGINAL PAPER

Emission of Volatile Aldehydes from DAG-Rich and TAG-Rich Oils with Different Degrees of Unsaturation During Deep-Frying

Izumi Katsuta · Masao Shimizu · Tohru Yamaguchi · Yoshinobu Nakajima

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Abstract The purpose of the present study was to compare the emission of volatile aldehydes from diacylglycerol-rich oils (DAG-OILs) and triacylglycerol-rich oils (TAG-OILs) with different degrees of unsaturation of fatty acid moieties during the deep-frying of sliced potatoes. To examine the effect of fatty acid composition, four kinds of oils with different fatty acid compositions were selected: rape seed (RS); sunflower oil as a high oleic (HO); safflower oil as high linoleic (HL); and, perilla oil as high linolenic (HLn) oils. The emissions of volatile aldehydes were determined during the deep-frying of sliced potatoes by using the above fresh test oils or deteriorated RS oils. The statistical analysis showed no significant difference in volatile aldehyde emission and profile between the DAG-OIL and TAG-OIL with the fatty acid composition of RS, HL, and HLn. Although a statistically significant difference was noted in the volatile aldehyde emission between the DAG-OIL and TAG-OIL with HO, this difference was extremely small when compared to the variations found in the oils with four types of fatty acid composition. Finally, no difference was found in the volatile aldehyde emissions between the deteriorated DAG-OIL and TAG-OIL, although volatile aldehyde emissions increased with frying time. In addition, the acrylamide contents in potato chips prepared with RS–DAG or RS–TAG were at comparable levels.

I. Katsuta · M. Shimizu (✉) · T. Yamaguchi · Y. Nakajima
Global R&D - Health Care Food, Kao Corporation,
2-1-3, Bunka, Sumida-ku, Tokyo 131-8501, Japan
e-mail: shimizu.masao@kao.co.jp

I. Katsuta
e-mail: katsuta.izumi@kao.co.jp

Keywords Deep-frying · Diacylglycerol · Triacylglycerol · Volatile aldehydes · Fatty acid composition · Glyceride structure · Thermal deterioration · Acrylamide

Abbreviations

TAG-OIL	Triacylglycerol-rich oil
DAG-OIL	Diacylglycerol-rich oil
RS oil	Rape seed oil
HO oil	High oleic oil
HL oil	High linoleic oil
HLn oil	High linolenic oil

Introduction

It is well known that some types of odors produced by the heating of cooking oil to high temperatures during deep-frying worsen the cooking environment. Cooking oils deteriorate due to heat and oxygen during deep-frying. This deterioration is accompanied by the generation of volatile aldehydes, fatty acids and alcohols, as secondary oxidation products. Volatile aldehydes are known to comprise a significant portion of the odor from cooking oils [1]. In particular, acrolein, considered to be the primary source of unpleasant odor, is reported to have an adverse effect on human health [2]. Generation of acrolein has been reported via two pathways: either from glycerol or from fatty acids [2, 3].

Some studies reported that the generation of volatile aldehydes depends on the composition of the cooking oils [4, 5]. There are various kinds of cooking oil on the market, each of which has differing molecular structure in terms of fatty acid forms and glyceride structure. To optimize the

comfort of the cooking environment, it is important to understand how volatile aldehyde emissions may be changed by these differences in fatty acid and glyceride structure.

There are various types of cooking oils with different fatty acid compositions. For example, olive and rape seed oils are rich in oleic acid, while corn oil is rich in linoleic acid. Recent studies have suggested that the type of volatile aldehyde emitted through thermal degradation varies with the fatty acid composition of cooking oils [6].

In recent years, oils with a different glyceride structure have been marketed. In both Japan and the USA, diacylglycerol-rich oil (DAG-OIL) has gained attention for its unique properties in lessening the accumulation of body fat [8, 9]. Although DAG displays different physicochemical properties from TAG [10, 11], oxidative deterioration during high-temperature cooking is similar [12].

However, little information is available concerning the influence of the glyceride structure on volatile aldehyde emissions. At least one study [7] reports that the types and amounts of volatile aldehyde emission do not differ between DAG-OIL and TAG-OIL; for that study, however, the fatty acid compositions of the test oils were not rigorously identical. Furthermore, no research has yet been carried out to examine simultaneously the effects of both fatty acids and glyceride structures on such volatile aldehyde emissions. Simultaneous studies on the above two parameters (fatty acids and glycerides) are expected to gain insights into the basic pathways for generation of aldehydes, including acrolein, that adversely affect the cooking environment.

The purpose of this study was to investigate in detail the inter-relationships of fatty acids and glycerides pertaining to the production of volatile aldehyde emissions. In addition, the effect of the glyceride structure on volatile aldehyde emissions during deep-frying for extended periods of time was investigated, along with an analysis of acrylamide, which has been reported to promote health problems in recent years.

Experimental Procedures

Fresh Oils

TAG-OILs with four types of fatty acid composition were used: high oleic sunflower oil as a high oleic (HO) oil, safflower oil as a high linoleic (HL) oil, perilla oil as a high linolenic (HLn) oil, and rape seed (RS) oil. HO, HL and HLn oils were used as samples to examine the effect of fatty acid composition, and RS oil was used as an example of common cooking oil. DAG-OILs with equivalent fatty acid compositions to TAG-OILs were prepared as follows: first, the fatty acids used for DAG-Oils preparation were obtained by the hydrolysis of corresponding TAG-OILs in the presence of Lipase AY (Amano Enzyme lnc., Nagoya, Japan). Subsequently, DAG-OILs were prepared with the esterification of glycerol and the fatty acids obtained by using the immobilized 1,3-regioselective lipase, Lipozyme RM IM (Novozymes A/S, Bagsvaerd, Denmark) and purified further as reported previously [13]. Hence, to match the antioxidant tocopherol compositions among the eight oils prepared above, α-tocopherol (ET-840R; J-OIL MILLS Inc., Tokyo, Japan), mixed tocopherpol (MTS-60S; Archer Daniels Midland Co., Decatur, IL, USA) and δ-tocopherol (E-Mix D; Eisai Food & Chemical Co., Ltd., Tokyo, Japan) were added to the oils. Table 1 shows the composition of prepared oils.

Deteriorated Oils

The deteriorated oils of RS–DAG and RS–TAG were obtained by treatment of deep-frying over 8 h continuously. Deep-frying tests without fresh oil replenishment were performed under the conditions reported in a previous study [12]. Table 2 shows the composition of the deteriorated oils prepared.

Sampling of Volatile Aldehyde Emissions

Volatile aldehydes generated during deep-frying were trapped according to the method reported by Lin et al. [5]. Briefly, deep-frying was performed for 15 min. Three hundred grams of test oil was placed in a round bottom flask that was heated with a mantle heater set to 180 °C. Thirty grams of non-prefried frozen sliced potatoes was fried for 3 min per batch, and 2 batches were processed within 15 min. The oil temperature was kept within the rage of 150–185 °C, and the average temperature of the oils was 170 °C. The flask was covered with a hood during the deep-frying, in which we collected almost all volumes of vaporized air at a rate of 70 L-dry air/min. Partial collected air at a flow rate of 700 mL-dry air/min was flown through the cartridge sampler (Sep-Pak DNPH-Silica cartridges; Waters Corp., Milford, MA, USA) for 15 min where all carbonyl compounds were trapped and converted to stable derivatives with dinitrophenylhydrazine (DNPH)-coated silica gel. The upstream region from the DNPH cartridges, Sep-Pak Ozone Scrubber (Waters Corp., Milford, MA, USA) was connected in order to remove an inhibitory factor [14]. Collecting lines and cartridges were kept warm at 50 °C with the aim of preventing the condensation of moisture. Frying experiments were done three times for each oil.

Table 1 Glyceride composition, fatty acid composition, tocopherol content, peroxide value, and *p*-anisidine value of test oils

	DAG-OIL				TAG-OIL			
	RS	HO	HL	HLn	RS	HO	HL	HLn
Glyceride composition (%)								
Free FA	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0
MAG	0.5	0.6	0.0	0.2	0.0	0.0	0.0	0.0
DAG	82.7	88.3	89.3	90.5	0.9	1.4	0.9	0.5
TAG	16.7	11.0	10.6	9.2	99.1	98.6	99.1	99.5
Fatty acid composition (%)								
Palmitic	4.8	3.5	6.7	5.7	4.3	3.5	6.9	6.2
Stearic	2.0	3.1	2.2	2.2	1.8	3.1	2.2	2.2
Oleic	60.6	87.1	15.6	19.1	59.0	87.0	14.8	18.5
Linoleic	21.2	4.9	73.8	12.8	21.4	4.7	73.0	12.6
Linolenic	10.3	0.3	0.4	59.7	12.3	0.3	0.4	60.0
Others	1.1	1.2	1.3	0.4	1.3	1.3	2.6	0.4
Tocopherol content (mg/kg)								
α-	536	460	500	49	560	500	523	491
β-	20	18	16	18	85	28	24	15
γ-	727	879	874	890	781	871	794	813
δ-	208	169	156	154	178	213	166	190
Total	1,492	1,525	1,546	1,551	1,604	1,611	1,507	1,508
Peroxide value (meq/kg)								
	0.93	0.37	0.97	0.72	0.60	1.61	3.26	3.57
p-Anisidine value								
	5.6	3.4	14.0	15.0	4.5	3.4	21.7	16.0

Table 2 Glyceride composition, fatty acid composition, and *p*-anisidine value of deteriorated oils

	RS-DAG	RS-TAG
Glyceride composition (%)		
Free FA	0.4	0.1
MAG	2.1	0.0
DAG	81.0	2.2
TAG	16.5	97.7
Fatty acid composition (%)		
Palmitic	4.4	4.2
Stearic	2.2	2.0
Oleic	62.5	61.1
Linoleic	19.4	19.3
Linolenic	7.6	8.9
Others	4.0	4.5
p-Anisidine value	168	178

Determination of Aldehydes, Fatty Acids and Glycerides

Aldehydes-DNPH derivatives were eluted from cartridges with 5 mL acetonitrile over 5 min. After filtering (0.45 μm, Kurabo Industries Ltd., Osaka, Japan), each of the eluates were diluted to 5 mL with acetonitrile. Aldehydes derivatives were measured with a high performance liquid chromatography (HPLC) system (LaChrom Elite; Hitachi High-Technologies Corp., Tokyo, Japan) equipped with an auto-sampler (L-2200) and a pump (L-2130). The reverse-phase column (Cadenza(5) CD-18; 3 μm, 250 × 4.6 mm; Imtakt Corp., Kyoto, Japan) was used to separate aldehyde derivatives. The column was warmed to 40 °C with a column oven (L-2300) and connected with a UV/VIS detector (L-2420) at 360 nm to detect aldehyde derivatives. The eluent was acetonitrile/distilled water = 70/30 for an initial 3 min, and a linear gradient of 30–100% in acetonitrile for a subsequent 30 min, then 100% acetonitrile at a flow rate of 0.4 mL/min. The external standard method was applied to determine each aldehydes level. Twenty types of volatile aldehydes were measured in the present study: propanal (C3:0), acrolein (C3:1), butanal (C4:0), 2-butenal (C4:1), pentanal (C5:0), 2-pentenal (C5:1), hexanal (C6:0), 2-hexenal (C6:1), 2,4-hexadienal (C6:2), heptanal (C7:0), 2-heptenal (C7:1), 2,4-heptadienal (C7:2), octanal (C8:0), 2-octenal (C8:1), nonanal (C9:0), 2-nonenal (C9:1), 2,4-nonadienal (C9:2), 2-decenal (C10:1), 2,4-decadienal (C10:2), 2-undecenal (C11:1). Additionally, emission of total volatile carbonyl compounds was calculated from total

peak area, and was expressed as an acrolein equivalent. Glyceride composition were determined by methods reported by previous study [12]. AOCS methods were used to determine the fatty acid composition (Cd 1f-96), the peroxide value (Cd 8b-90) and the *p*-Anisidine value (Cd 3b-63).

Statistical Analysis

First, the total amounts of volatile carbonyl compounds obtained from the total peak area of all compounds derived by DNPH were analyzed. Effects of fatty acid composition, glyceride structure and their second order interaction were estimated by analysis of variance (ANOVA) in the linear model of Eq. (1).

$$\log(y) = \mu + a_i + b_j + (ab)_{ij} + e_{ij} \quad (1)$$

where μ, a_i, b_j, and $(ab)_{ij}$ are over all means, type of oil effect, structure of glyceride effect and interaction of a_i and b_j respectively; $e_{ij} \sim N(0, \sigma^2)$ is residual error; and i represents one of (RS, HO, HL, HLn), j represents one of (DAG, TAG). The total amounts of volatile carbonyl compounds, y were logarithmically transformed to normalize the data.

Second, volatile aldehydes from each oil were analyzed. The effects of fatty acid composition, glyceride structure, forms of aldehydes and their second order interactions were estimated by ANOVA in the linear model of Eq. (2).

$$\log(y) = \mu + a_i + b_j + c_k + (ab)_{ij} + (bc)_{jk} + (ac)_{ik} + e_{ijk} \quad (2)$$

where μ, a_i, b_j, c_k, $(ab)_{ij}$, $(bc)_{jk}$ and $(ac)_{ik}$ are overall means, type of oil effect, structure of glyceride effect, aldehyde effect, interaction of a_i and b_j, interaction of b_j and c_k, interaction of a_i and c_k respectively; $e_{ijk} \sim N(0, \sigma^2)$ is residual error; and i represents one of (RS, HO, HL, HLn); j represents one of (DAG, TAG); k represents kind of aldehydes ($k = 1, ..., 20$).

In addition to the above, a long-duration frying test of RS oil was analyzed using ANOVA with the linear model of Eq. (2), where a_i is fresh oil or deteriorated oil.

Finally, aldehydes from each of the fatty acids in oils were calculated and analyzed. To determine the relationship between aldehydes from each of the fatty acids and each fatty acid concentration in oils, the parameters in a linear structural relationship line were estimated considering variances of thrice-replicated measurements. Confidence interval was estimated by the bootstrap BC method [15, 16], where re-sampling times were 100,000.

Two-tailed tests were used and $p < 0.05$ was considered as statistically significant throughout the analysis. The analysis was performed with SAS release 8.2 (SAS Institute Inc., Cary, NC, USA).

Determination of Acrylamide Content

Potato chip frying tests were done with RS-DAG and RS-TAG. Sliced potatoes (May Queen, 1 mm thick) were fried in 400 g of oil. The frying time per batch was 2 min, and 4 batches (30 g/batch) were processed in 15 min. Acrylamide content in potato chips and oils after the frying test were measured with GC–MS at the Japan Food Research Laboratories according to the method reported by Yamazaki et al. [17].

Results and Discussion

First, the total amounts of volatile carbonyl compounds generated during deep-frying were compared. Because of asymmetric distribution of the crude data due to the measurement system, calculation of averaged values and statistical analysis were carried out after normalization of data by logarithmic transformation. Table 3 shows the averages of total amounts of volatile carbonyl compounds generated from each oil. There were statistically significant differences in the production of volatile carbonyl compounds among the oils with four types of fatty acid compositions ($p < 0.001$). However, for each of the four types of oil, there were no significant differences between the oils with different glyceride structures ($p = 0.616$). These results indicate that the total amounts of volatile

Table 3 Emission of volatile carbonyl compounds normalized by the amount of acrolein

	Emission of volatile carbonyl compounds[a] (μmol-acrolein-equivalent/kg-lipid/h)			
	NS			
	DAG-OIL ($N = 12$)		TAG-OIL ($N = 12$)	
	Mean	+SE −SE	Mean	+SE −SE
RS oil ($N = 3$)	3,107	3,832	2,453	3,379
#		2,519		1,780
HO oil ($N = 3$)	705	1,106	820	883
		450		762
HL oil ($N = 3$)	1,945	2,013	2,602	2,800
		1,879		2,417
HLn oil ($N = 3$)	4,830	4,939	5,356	5,608
		4,722		5,115

NS not significant

# $P < 0.001$

[a] Data were calculated from Eq. (1), and expressed as the geometric mean ± standard error; SE. There were significant differences among the oils ($p < 0.001$), and was no significant difference between DAG-OILs and TAG-OILs ($p = 0.616$)

carbonyl compounds produced were not affected by the glyceride structure, but solely by their original fatty acid composition.

Individual comparison of the volatile carbonyl compounds was considered. Figure 1 shows volatile aldehyde emissions from each oil. Comparing DAG-OIL and TAG-OIL, the plots nearly overlapped overall even though there was a little difference in the long-chain aldehydes. This result suggests that both the amount and the composition of volatile aldehydes in DAG-OIL and TAG-OIL were almost identical. In contrast, the patterns of aldehydes were completely different among the four types of oils with different fatty acid compositions. It is known that the type of aldehyde generated is determined by the fatty acids through oxidization. The primary products of aldehydes from each of the test oils found in the present study were consistent with previous studies [18, 19]. In contrast, no differences were found in the production of volatile aldehydes between DAG-OIL and TAG-OIL. These results strongly suggest that the pattern of volatile aldehydes emissions was influenced by fatty acid pattern rather than by glyceride structure.

In order to compare the emission of volatile aldehydes from the major fatty acids that make up DAG-OIL and TAG-OIL, the linear correlation between contents of oleic acid, linoleic acid or linolenic acid in each of the oils was examined, along the amounts of aldehyde production. Separated analysis of DAG-OIL or TAG-OIL indicated that some of the aldehydes were highly correlated with a certain type of fatty acid content. The aldehydes with high correlation ($r > 0.8$, $p < 0.05$) showed a consistent pattern between DAG-OIL and TAG-OIL. The following are the aldehydes in which the productions were well-correlated with certain types of fatty acid contents: Octanal (C8:0), Nonanal (C9:0) with oleic acid; Pentanal (C5:0), Hexanal (C6:0), 2-Heptenal (C7:1), 2-Octenal (C8:1), 2-Nonenal (C9:1), 2,4-Nonadienal (C9:2), 2,4-Decadienal (C10:2) with linoleic acid; Propanal (C3:0), Acrolein (C3:1), 2-Butenal (C4:1), 2-Pentenal (C5:1), 2,4-Hexadienal (C6:2), 2,4-Heptadienal (C7:2) with linolenic acid. Most of the correlations listed above are consistent with results reported previously [1]. For these aldehydes, volatile aldehyde productions per 1 kg of fatty acid were calculated based on TAG-OIL and DAG-OIL (Fig. 2). Although the correlation between octanal and DAG-OIL was slightly low ($p = 0.075$), octanal was included in this plot, taking its low aldehyde production into consideration. A linear structural relationship line $E(y) = \alpha + \beta E(x)$ was estimated where $E(y)$, is the expectation value of amount of aldehydes per 1 kg of fatty acid from DAG-OIL, and $E(x)$ is the expectation value from TAG-OIL. Parameters were $\alpha = -0.050$, $\beta = 0.94$, and the null hypothesis that $E(x)$ was equal to $E(y)$ could not be rejected. As a result, this linear relationship suggests that volatile aldehydes emitted per weight of fatty acid were not significantly different between DAG-OIL and TAG-OIL.

As described earlier, previous studies reported two pathways in the generation of acrolein from glycerol and fatty acids [2, 3]. In the present study, the amount of acrolein generated was dependent on the content of

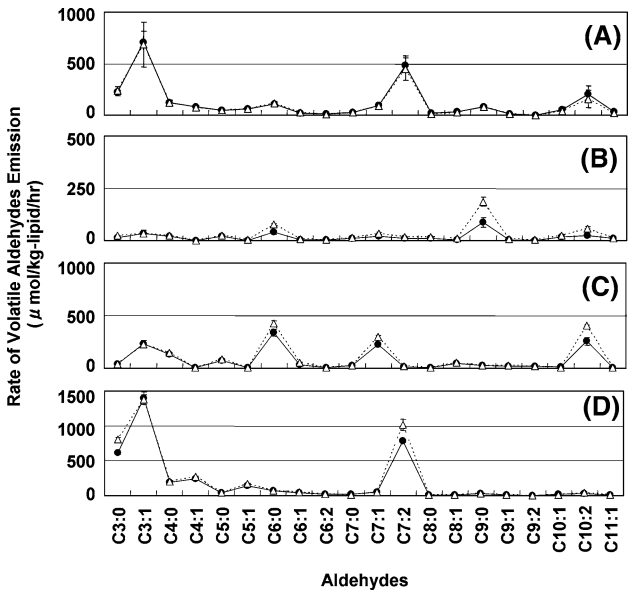

Fig. 1 Emission of 20 volatile aldehydes from RS oils (**a**), HO oils (**b**), HL oils (**c**) and HLn oils (**d**) of DAG-OIL (*filled circles*) and TAG-OIL (*open triangles*). Data represent mean ± SE

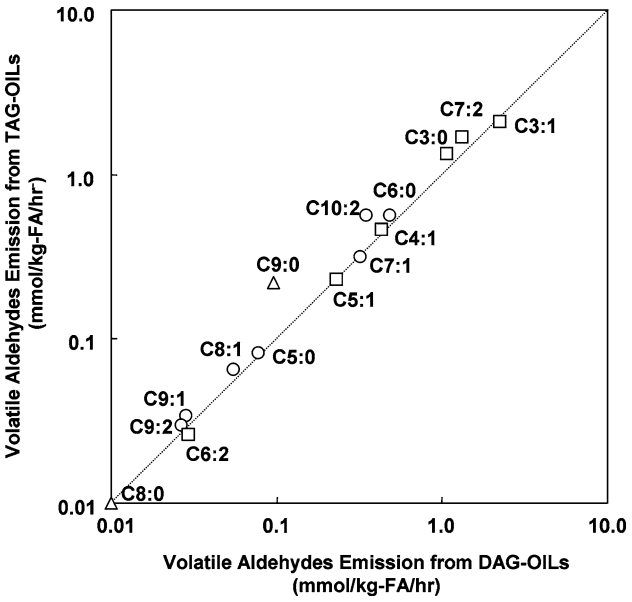

Fig. 2 Volatile aldehydes emission per 1 kg of Oleic acid (*open triangles*), Linoleic acid (*open circles*), or Linolenic acid (*open squares*) in DAG-OILs or TAG-OILs

linolenic acid. These results suggest that the dominant pathway for acrolein generation is from fatty acids, and in particular from linolenic acid, rather than from glycerol. In fact, the weight ratio of glycerol in the DAG molecule is calculated about 1.4 times higher than that in TAG molecules, because of the difference in the number of fatty acid moieties [20].

As shown in Table 4, the ANOVA showed a significant effect due to the fatty acid composition, and for the glyceride structure only in the HO-OILs. However, compared with the averages of total emissions from each oil (Table 4 right), the differences between HO-DAG and HO-TAG were extremely small when compared to the variation correlated to fatty acid composition. Meanwhile, it was confirmed that the amounts of total carbonyl compounds accumulated in oils were almost equivalent between HO-DAG and HO-TAG as determined with the measurement of a *p*-anisidine value (9.0 ± 2.0 and 10.7 ± 1.7 ppm, respectively). The results showed that the amount of volatile aldehydes produced differ greatly by fatty acid composition, rather than by a distinction of glyceride structure in RS oil, HL oil and HLn oil, despite a slight difference in HO oil.

For the present study, the production of acrylamide generated during high-temperature cooking (e.g. deep-frying of potato chips) was examined. Acrylamide has been shown to cause health problems in recent years. The results indicated that the content of acrylamide in potato chips cooked with DAG-OIL and TAG-OIL were 0.7 ± 0.3 and 1.3 ± 0.4 ppm, respectively, suggesting comparable levels. Acrylamide in both oils after a frying test was not detected. It is known that acrylamide is generated by the reaction of amino acids with sugar included in potatoes [21, 22]. The present results showed that cooking oils with different glyceride structure did not affect the production of acrylamide.

Finally, volatile aldehydes emissions during extended frying are shown in Fig. 3. There was no significant difference in volatile aldehydes emissions between deteriorated RS-DAG and RS-TAG after frying for a long period ($p = 0.584$). Significant increases in aldehyde production were found from deteriorated oils compared to fresh oils in both RS-DAG ($p < 0.001$) and RS-TAG ($p < 0.001$). The increase in aldehyde production was more evident from medium-chain to long-chain aldehydes. This is because medium-chain and long-chain aldehydes evaporated less, due to a higher boiling point, thereby accumulating in the cooking oil after extended cooking periods. In contrast, production of some types of aldehyde derived from linolenic acid (e.g. acrolein) decreased during cooking. Actually, the contents of linolenic acids in deteriorated RS-DAG and RS-TAG decreased by 14 and 15% compared to fresh oil, respectively. A lower production of aldehydes derived from linolenic acid is explained by the reduced contents of linolenic acid in oils that are easily oxidized during cooking. As a result, the production of aldehydes did not differ between deteriorated RS-DAG and RS-TAG, although an increased production of aldehydes was noted from deteriorated oils compared to fresh ones.

This finding is consistent with results reported by Li et al. [7], in terms of similar production of aldehyde between DAG-OIL and TAG-OIL. However, sensory evaluations from a prior study differ from the present one in that irritating odor was not recognized from DAG-OIL in the latter. Although the reason for this difference is uncertain, it could be that fatty acid composition between DAG-OIL and TAG-OIL were not rigorously identical in the previous study. In addition, free fatty acid content in

Table 4 Statical analyses regarding the total emission of 20 volatile aldehydes

	DAG-OIL/ TAG-OIL ratio[a]		Total emission (μmol/kg lipid/h)			
	Mean	*p* Value	DAG-OIL		TAG-OIL	
			Mean	+ SE −SE	Mean	+ SE −SE
RS oil ($N = 60$)	1.17	0.060	3,141	3,755	2,696	3,266
				2,627		2,225
HO oil ($N = 60$)	0.48	<0.001	395	486	825	981
				320		693
HL oil ($N = 60$)	0.90	0.215	1,893	2,260	2,094	2,536
				1,586		1,729
HLn oil ($N = 60$)	1.06	0.456	2,948	3,630	2,774	3,500
				2,394		2,199

Data were calculated from Eq. (2), and expressed as the geometric mean of DAG-OIL/TAG-OIL Ratio, as the geometric mean ± standard error; SE in Total Emission

[a] DAG-OIL/TAG-OIL Ratio = exp [log (total emission from DAG-OIL) − log (total emission from TAG-OIL)]

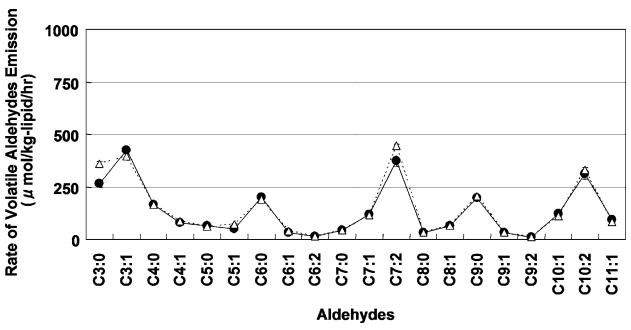

Fig. 3 Volatile aldehydes emission from deteriorated RS-DAG (*filled circles*) and deteriorated RS-TAG (*open triangles*). Data represent means ± SE

DAG-OIL was higher than that of TAG-OIL, which may affect sensory evaluation tests.

In summary, the amount and composition of volatile aldehydes produced during frying were not affected by the glyceride structure, but solely by their original fatty acid composition. Consequently, it was determined that the mechanism of oxidative deterioration during cooking was the same between oils with different glyceride structures. And there was no difference when oils had deteriorated from long-period frying. Accordingly, it was concluded that generational patterns for aldehyde production were the same between DAG-OIL and TAG-OIL, even when used for long-period frying.

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