Chiral separation of triacylglycerol containing acetyl group in fully hydrogenated plant oil

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INTRODUCTION Edible oils and fats are mainly composed of triacylglycerols (TAGs). In another presentation (1) by our research group at this congress, LC/MS measurements (**Fig. 1a, 1b**) revealed that trace amounts of TAG containing acetyl group such as dioleoyl acetylglycerol (OOAc) are present in common edible oils. OOAc in edible oils shown in **Table 1** was quantified using a standard *sn*-OOAc via LC/MS. For other components, calculated values were shown using the OOAc calibration curve (data not shown).

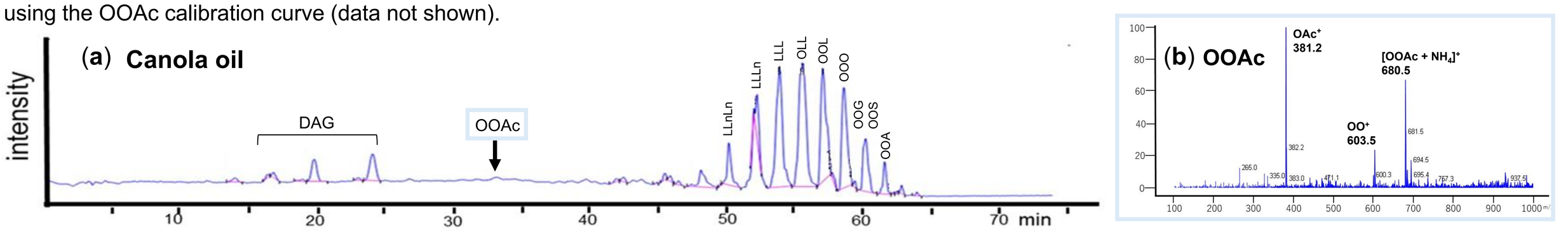

Fig. 1 TIC chromatogram of canola oil TAGs (a) and mass spectrum of dioleoyl acetylglycerol (OOAc) in canola oil (b). HPLC Conditions; Column: Inertsil ODS-3 (250 mm x 2.1 mm i.d., 3 µm, GL Sciences Inc.), Column temperature: 40˚C, Mobile phase: acetone / methanol 0/100 (0 min) →0/100 (30 min) → 100/0 (60 min) , Flow rate: 0.2 mL/min, MS conditions; System: G6135B Single Quad LC/MSD (Agilent Technologies, Inc.), Ionization: ESI-positive (0.1 mL/min of 100 mM ammonium formate methanol solution was mixed at a post-column position to promote ionization), Scan mode (*m/z* 100-1000).

However, the ratio of *sn*-OOAc/*sn*-AcOO/*sn*-OAcO is unknown in natural oils, and the reverse-phase (RP) HPLC used in the analysis shown in **Fig. 1** cannot separate these isomers. LC/MS analysis requires separation of TAG positional isomers such as *sn*-OOAc/*sn*-OAcO due to their differing calibration curve slopes. Furthermore, the binding position of the acetyl group—whether at the *sn*-1 or *sn*-3 position— seems to be biologically significant, requiring the chiral separation of enantiomers such as *sn*-OOAc/*sn*-AcOO by chiral HPLC. However, it is unclear whether OOAc can be separated using the previously reported chiral HPLC method (2). Therefore, in this study, fully hydrogenated plant oils were used as the sample, and chiral HPLC analysis was performed targeting SSAc, the hydrogenated form of OOAc, OLAc, OLnAc, LLAc, LLnAc, etc. (**Fig. 2**).

Table 1 TAGs containing acetyl group in edible oils and fats (mg/kg oil)

	Canola	Canola (unrefined)	Soybean	Corn	Rice	Olive	Palm	Lard
OOAc	**207**	**195**	**39**	**11**	**27**	**38**	**40**	**5**
PPAc					*9*	*5*		*27*
POAc	*20*	*17*	*23*	*5*	*10*	*9*	*84*	*5*
OLAc	*142*	*124*	*106*					
OLnAc	*33*	*26*	*11*					
LLAc	*60*	*49*	*192*	*12*	*13*		*8*	*2*
LLnAc	*29*	*23*	*52*					
TOTAL	*491*	*434*	*423*	*27*	*58*	*51*	*633*	*38*

OOAc was quantified using a standard (*sn*-OOAc, Larodan AB). For other components, calculated values are shown using the *sn*-OOAc calibration curve.

EXPERIMENTAL

Materials: Fully hydrogenated canola oil (FHCO), soybean oil (FHSO), and palm oil (FHPO) were obtained from a domestic manufacturer in Japan. A Interesterified oil of triacetin (AcAcAc)-FHCO (SSS) (90:10, w/w) was synthesized using sodium methoxide as a standard for detecting SSAc by MS.

Preparative RP-HPLC: SSAc-containing fractions (RT 4-28 min) that eluted before long-chain trisaturated TAGs in fully hydrogenated oils were collected using preparative RP HPLC (**Fig. 3**). Column: Inertsil ODS-HL (250 mm x 20 mm i.d., 5 µm, GL Sciences Inc.), Column temperature: 40˚C, Mobile phase: methanol / chloroform 70/30 (v,v), Flow rate: 20 mL/min, Injection volume: 1 mL × 10% fully hydrogenated oil in chloroform. The fractions containing SSAc were analyzed using an analytical-scale HPLC-RI system, confirming it contained no SSS.

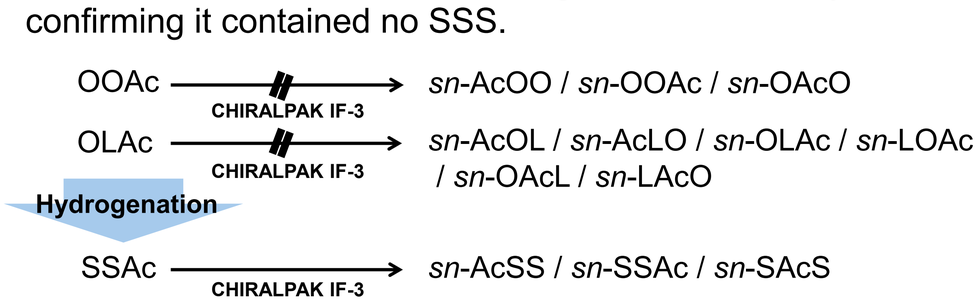

Fig. 2 Prediction of the effect of hydrogenation on the chiral HPLC separation behavior of TAG molecular species

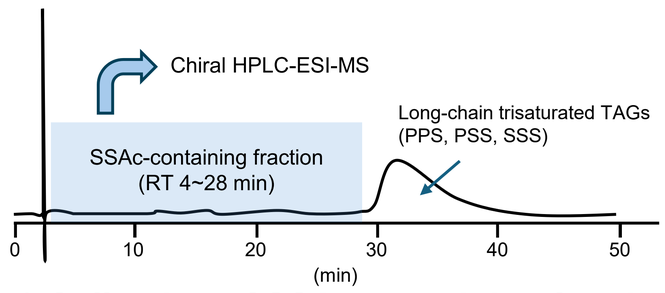

Fig. 3 Collection of SSAc-containing fraction in fully hydrogenated oils by preparative RP-HPLC

Chiral HPLC-ESI-MS: The SSAc-containing fractions were analyzed by chiral HPLC and ESI-MS. Column: CHIRALPAK IF-3 (250 mm x 2.1 mm i.d., 3 µm, Daicel Corporation), Column temperature: 25˚C, Mobile phase: acetonitrile, Flow rate: 0.2 mL/min; Injection volume: 5 µL × 0.1% SSAc-containing fraction. MS System: G6135B Single Quad LC/MSD (Agilent Technologies, Inc.), Ionization: ESI-positive mode (0.1 mL/min of 100 mM ammonium formate methanol solution was mixed at a post-column position to promote ionization), SIM (*m/z* 684) corresponding to $[SSAc+NH_4]^+$.

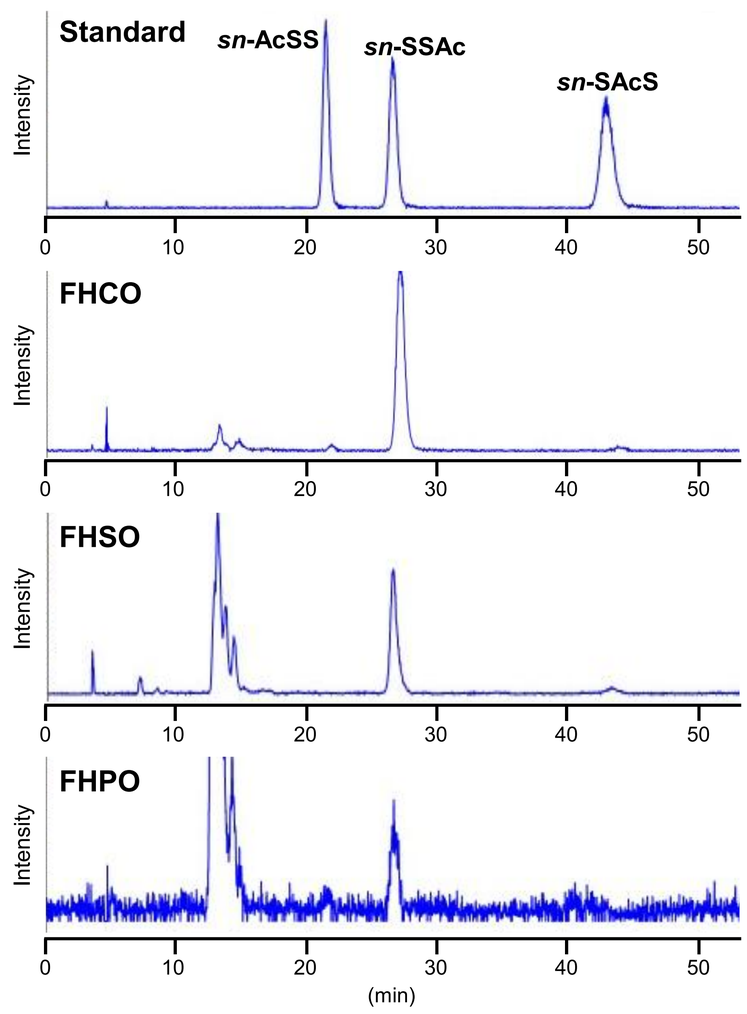

Fig. 4 SIM chromatograms of SSAc (*m/z* 684) of standard containing all isomers of SSAc (interesterified oil of triacetin and FHCO), FHCO, FHSO, and FHPO on CHIRALPAK IF-3

RESULTS AND DISCUSSION

To analyze the binding position of the acetyl group in TAGs in the above plant oils, FHCO, FHSO, and FHPO were subjected to chiral HPLC equipped with CHIRALPAK IF-3. The reasons for using fully hydrogenated oils were that TAG molecules composed of unsaturated fatty acids with 18 carbon atoms and acetyl groups (OOAc, OLAc, LLAc, OLnAc, LLnAc, LnLnAc) are all converted to SSAc by hydrogenation (**Fig. 2**) and that our previous study (2) had shown that CHIRALPAK IF-3 successfully separate TAG isomers composed of P and O eluting in order of *sn*-OPO < *sn*-OOP < *sn*-POO < *sn*-OPP < *sn*-PPO < *sn*-POP. When SSAc is chromatographed on CHIRALPAK IF-3, the isomers are probably eluted in order of *sn*-AsSS < *sn*-SSAc < *sn*-SAcS because the isomers having long-chain saturated fatty acids on a glycerol backbone are strongly retained in the order *sn*-1 > *sn*-3 > *sn*-2. As the results of chiral HPLC, three peaks were detected in the chromatogram of the standard, each of which was presumed to be *sn*-AcSS, *sn*-SSAc, and *sn*-SAcS, respectively (**Fig. 4**). Furthermore, *sn*-SSAc was the main component in the SIM chromatograms (*m/z* 684) of fully hydrogenated oils analyzed. These results suggest that acetyl groups are selectively bound to the *sn*-3 position in TAGs in the oils and fats before hydrogenation.

ABBREVIATIONS: Ac = acetic acid (C2:0), P = palmitic acid (C16:0), O = oleic acid (C18:1), L = linoleic acid (C18:2), Ln = linolenic acid (C18:3), A=Arachdic acid (C20:0), G=Gadoleic acid (C20:1). Prefix, '*sn*-' indicates that the fatty acid binding positions on the glycerol backbone of TAG are fixed. For example, *sn*-PPO indicates 1,2-dioleoyl-3-palmitoyl-*sn*-glycerol.

REFERENCES

1) M. Amano et al., Triacylglycerol containing acetyl group in edible oil, Analytics, Authenticity, Contaminants, *Euro Fed Lipid 2025* (**Tue, 14 Oct, Afternoon**)

2) T. Nagai et al., Separation of triacylglycerol enantiomers and positional isomers by chiral high performance liquid chromatography coupled with mass spectrometry, *J. Oleo. Sci.* 68, 1019-1026 (2019).