Regiospecific Distribution of trans-Octadecenoic Acid Positional Isomers in Triacylglycerols of Partially Hydrogenated Vegetable Oil and Ruminant Fat

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Abstract: It is revealed that binding position of fatty acid in triacylglycerol (TAG) deeply relates to the expression of its function. Therefore, we investigated the binding positions of individual trans-octadecenoic acid (trans-C18:1) positional isomers, known as unhealthy fatty acids, on TAG in partially hydrogenated canola oil (PHCO), milk fat (MF), and beef tallow (BT). The analysis was carried out by the sn-1(3)-selective transesterification of Candida antarctica Lipase B and by using a highly polar ionic liquid capillary column for gas chromatography–flame ionization detection. trans-9-C18:1, the major trans-C18:1 positional isomer, was selectively located at the sn-2 position of TAG in PHCO, although considerable amounts of trans-9-C18:1 were also esterified at the sn-1(3) position. Meanwhile, trans-11-C18:1, the major isomer in MF and BT, was preferentially located at the sn-1(3) position. These results revealed that the binding position of trans-C18:1 positional isomer varies between various fats and oils.

Key words: trans fatty acid, triacylglycerol, hydrogenated fat, milk fat

1 INTRODUCTION

Triacylglycerol (TAG) is a major component of edible fats and oils and consists of one glycerol moiety and three fatty acids. The binding positions of the fatty acids can be categorized into two types, with the primary and secondary alcohol groups on the glycerol being defined as the sn-1 (3) and sn-2 positions, respectively. The specific position to which fatty acids bind affects their absorption and function in both animals and humans. For instance, palmitic acid (C16:0) is found at the sn-2 positions on TAG in the human milk, as this allows infants to get energy more easily [1,2]. Likewise, in fish oil, n-3 series polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid and docosahexaenoic acid (DHA), are mainly esterified at the sn-2 position; however, n-3 PUFAs in the seal oil are mainly located at the sn-1(3) positions [3,4]. Recently, we compared the effects of the binding positions of n-3 PUFAs on lipid metabolism, and observed that DHA bound to the sn-2 position of TAG.
sition inhibited fatty acid synthetic enzymes more effectively than DHA bound to the sn-1(3) position, thereby reducing liver TAG concentrations\(^6\). In this way, the fatty acid binding position can have a positive effect on the human health. However, the effects of the binding position on unhealthy fatty acids like trans fatty acids (TFAs) have not yet been studied.

A high TFA intake is known to increase the risk of cardiovascular diseases (CVD)\(^6,7\). Unfortunately, TFA is found in industrially produced partially hydrogenated vegetable oil (PHVO), and occurs naturally in ruminants such as cows, sheep, and goats due to biohydrogenation caused by anaerobic fermentation in the rumen. Furthermore, despite widespread consumption, very few studies have investigated the TAG-binding positions of TFA in milk fat (MF). Woodrow et al. used infrared spectroscopy to determine positional distribution, yet observed no measurable amounts of TFA in isolated 2-monooacyl-sn-glycerol (2-MAG) from MF\(^8\). Reports by Barbano & Sherbon\(^9\) and Parodi\(^10\) agree with this result, showing that most TFA is located at the sn-1(3) position; however, small amounts of TFA were also found at the sn-2 position. Recently, Kallio et al. suggested that the binding position of TFA depends on the two other fatty acids present on TAG\(^11\).

Most of the previous studies have determined the binding position of TFA in ruminant fats, yet did not account for TFA positional isomer. In both PHVO and ruminant fats, TFA is mainly composed of approximately 80-90% trans-octadecenoic acid (trans-C18:1)\(^12\); the exact proportion of positional isomers is dependent on the kind of fat in question. For example, PHVO characteristically contains a high percentage of elaidic acid (trans-9-C18:1) and trans-10-C18:1\(^13\). In contrast, TFA in ruminant fats is primarily vaccenic acid (trans-11-C18:1)\(^14,15\). Epidemiological studies have shown that a high PHVO intake is associated with the increased risk of CVD because of the effects of TFA on the lipoproteins in plasma\(^16\); in contrast, no positive association between the ruminant fat and CVD has been reported\(^17,18\). These contrary health effects may be caused by the different distributions of trans-C18:1 positional isomers, though it is still not clear how specific trans-C18:1 positional isomers influence the human health because it is difficult to obtain, distinguish, and quantify them by conventional analytical methods\(^19-21\). To overcome these problems, we recently developed a new analytical method that provides good resolution for all potential isomers using a highly ionic liquid capillary column for gas chromatography–flame ionization detection (GC-FID)\(^22\).

The most widely used method for analyzing the binding positions of fatty acids on TAG is enzymatic, and is based on sn-1(3) selective hydrolysis using pancreatic or microbial lipase\(^23,24\). However, these methods cannot be applied to TAG containing short chain fatty acids (SCFAs) or PUFAs due to the fatty acid selectivity of the lipases. In recent years, a new enzymatic method using immobilized Candida antarctica Lipase B (CALB) has been applied to these compounds\(^25\); nevertheless, the extent to which differences in the cis and trans isomerism affect this enzymatic reaction, if at all, is not yet known.

In the present study, we used structured lipids consisting of two oleic acid cis-9-C18:1 moieties and one trans-9-C18:1 moiety to further study this topic. In addition, the total fatty acid content of PHVO and ruminant fat, with a specific focus on trans-C18:1 positional isomers, was determined in order to elucidate the regiospecific distribution of TFAs in various TAGs.

2 EXPERIMENTAL

2.1 Chemicals and materials

Canola oil (CO), partially hydrogenated canola oil (PHCO), MF, and beef tallow (BT) were in-house products (Tsukishima Foods Industry Co., Ltd., Tokyo, Japan). The structured lipids, 1,3-dioleoyl-2-elasfoyd-sn-glycerol (OEO; purity, 99%); and 1,2-dioleoyl-3-elaidoyl-rac-glycerol (OOL; purity, 99%), immobi-lized CALB (Chirazyme L-2 C4), and other reagents were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

2.2 Evaluation of sn-1(3) selective transesterification using structured lipids

2.2.1 Enzymatic reaction of TAG

The regiospecific distribution of fatty acids in the structured lipids was determined following a procedure developed by Watanabe et al.\(^26\). First, 0.5 g of lipid was treated with 5.0 g of ethanol and 0.33 g of Chirazyme L-2 C4. The reaction mixture was shaken in a 30°C water bath for 3 h at 150 rpm, after which the solution was checked by thin layer chromatography (TLC) on silica gel with a mixed mobile phase of chloroform, acetone, and acetic acid (96:4:1, v/v/v). Next, 0.1 mL of the resulting sample lipid was loaded into a Sep-Pak Silica cartridge (0.65 g, Waters Corporation, Milford, MA, USA) that had been pre-equilibrated using a solvent mixture of hexane and diethyl ether (80:20, v/v). The fatty acid ethyl ester (FAEE) was then collected by eluting with 10 mL of the same solvent mixture; an additional 20 mL of the solvent mixture was passed through to remove diacylglycerol. Finally, the 2-MAG was collected using 10 mL of diethyl ether. To prepare fatty acid methyl esters (FAMEs) from 2-MAG fraction, diethyl ether was removed by evaporation. FAME fraction was directly analyzed by GC-FID.

2.2.2 GC analysis of fatty acid composition

TAG and the enzymatically prepared 2-MAG were converted to FAMEs according to a modified version of American Oil Chemists’ Society (AOCS) method Ce 1b-89\(^27\). A
100 mg sample was mixed with 1 mL of a 5 mg/mL solution of triheneicosanoyl-glycerol in chloroform, which served as an internal standard. After solvent evaporation, the sample was saponified in 1.5 mL of a 0.5 N solution of sodium hydroxide in methanol by heating at 100°C for 9 min. The resulting solution was added to 2 mL of a 14% solution of boron trifluoride in methanol and further incubated at 100°C for 7 min. After the addition of 1 mL of hexane and 5 mL of saturated sodium chloride solution, the upper hexane layer was extracted and dried over anhydrous sodium sulfate.

The fatty acid composition was quantified according to AOCS method Ce 1h-05\(^{13}\). This was achieved using a GC-FID (TRACE GC Ultra, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a capillary column (SP-2560, 100 m × 0.25 mm i.d., 0.20 μm thickness, Sigma-Aldrich Japan K.K., Tokyo, Japan). The temperatures of both the injection port and detector were maintained at 250°C. The column temperature was maintained at 180°C and the split ratio was 100:1. The flow rate of the helium carrier gas was 1.0 mL/min.

2.3 Analysis of edible fats and oils

2.3.1 Regiospecific distribution of fatty acids in edible fats and oils

The enzymatic reaction of CO, PHCO, MF, and BT was conducted by the method detailed in section 2.2.1. The fatty acid composition of TAG and 2-MAG samples from CO, PHCO, MF, and BT was determined by AOCS method Ce 1h-05\(^{19}\), which is capable of separating the geometrical isomers of fatty acids (Section 2.2.2).

In order to investigate the preferential binding tendencies of fatty acids to TAG, the regiospecificity of each fatty acid at the sn-2 position (mol/100 mol) was calculated from the following equation:

\[ \text{Regiospecificity} = \frac{F_{\text{sn-2}}}{F_{\text{sn-1}}} \times 100 \]

where \( F_{\text{sn-2}} \) and \( F_{\text{sn-1}} \) are the fatty acid contents (g/100 g oil) of 2-MAG and TAG, respectively. We defined 33.3 mol/100 mol as the regiospecific value at which the fatty acid is equally distributed because, if fatty acids were evenly distributed between all sn-positions, a 33.3% regiospecificity would be observed for the sn-2 position. As such, higher values indicate preferential binding at the sn-2 position, while lower values suggest stronger binding tendencies for sn-1 (3).

2.3.2 Regiospecific distribution of trans-C18:1 positional isomers

In order to determine the contents of trans-C18:1 positional isomers in TAG and 2-MAG samples from PHCO, MF, and BT, the trans-C18:1 fractions in FAMEs of TAG and 2-MAG samples were separated from other fatty acids following a procedure developed by Goto et al.\(^{27}\). First, a 0.5 mg sample of the produced FAME was loaded into a silver-ion cartridge (Ag-ION SPE, Discovery Ag-ION, Sigma-Aldrich Japan K.K., Tokyo, Japan) that had been pre-equilibrated by 4 mL of acetone and then 8 mL of hexane. Then, the saturated fatty acid fraction was removed using 6 mL of dichloromethane, after which the trans-C18:1 fraction was collected by eluting with 6 mL of dichloromethane/ethyl acetate (90:10 v/v). After solvent evaporation, the residue was dissolved in a small volume of hexane for subsequent GC-FID analysis.

Determination of the individual trans-C18:1 positional isomers was accomplished by a GC-FID system (TRACE GC Ultra) equipped with an SLB-IL111 capillary column (100 m × 0.25 mm i.d., 0.20 μm thickness, Supelco, Bellefonte, PA, USA). This procedure was based on the authors’ previous method\(^{22}\). Both the injector and detector temperatures were set to 250°C. The column temperature was maintained isothermally at two different temperatures (120 and 160°C). The split ratio was 100:1 and the flow rate of the helium carrier gas was 1.2 mL/min.

The regiospecificity of each trans-C18:1 positional isomer at the sn-2 position was calculated by the method detailed in section 2.3.1.

2.4 Statistical analysis

Each value is presented as mean ± SE. Student’s t-test was employed to detect the differences between 33.3 mol/100 mol as a theoretical value with the experimental regiospecific values of each fatty acid at the sn-2 position. \( P<0.05 \) was considered statistically significant.

3 RESULTS AND DISCUSSION

3.1 Evaluation of sn-1 (3) selective transesterification using structured lipids

The selective sn-1 (3) transesterification of TAG by CALB was initially evaluated using structured lipids as substrates. The enzymatic reaction was conducted at 30°C for 3 h; TLC was used to confirm the total reaction of TAG. The FAEE and 2-MAG fractions were isolated by silica gel chromatography, after which their fatty acid composition was analyzed by GC-FID (Table 1). If direct sn-2 transesterification or acyl migration from sn-2 to sn-1 had not occurred, only cis-9-C18:1 would have been detected in the OEO FAEE fraction. In the same manner, cis-9-C18:1 and trans-9-C18:1 would have been seen in equal concentrations in the OEE FAEE fraction. However, the OEO FAEE fraction showed a trans-9-C18:1 concentration of 3.7 mol%, while the OEE FAEE fraction gave cis-9-C18:1 in a higher proportion. In contrast, we found these effects to be minor for the 2-MAG fraction; cis-9-C18:1 content in the OEO-derived fraction and trans-9-C18:1 content in the OEE-derived fraction were both less than 1%. These findings are in agreement with previous reports by Watanabe.

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\( ^{13} \text{J. Oleo Sci. 64, (6) 617-624 (2015)} \)
Table 1  Fatty acid compositions (mol%) of FAEE and 2-MAG fractions from OEO and OOE after sn-1(3)-selective transesterification.

<table>
<thead>
<tr>
<th></th>
<th>FAEE fraction</th>
<th>2-MAG fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cis-9-C18:1</td>
<td>trans-9-C18:1</td>
</tr>
<tr>
<td></td>
<td>cis-9-C18:1</td>
<td>trans-9-C18:1</td>
</tr>
<tr>
<td>OEO</td>
<td>96.2 ± 0.2</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>OOE</td>
<td>54.8 ± 0.1</td>
<td>45.1 ± 0.0</td>
</tr>
</tbody>
</table>

Each value represents mean ± SE (n=3).
FAEE, fatty acid ethyl ester; 2-MAG, 2-monoacyl-sn-glycerol; OEO, 1,3-dioleoyl-2-elaidoyl-sn-glycerol; OOE, 1,2-dioleoyl-3-elaidoyl-rac-glycerol (OOE).

Table 2  Regiospecific analysis of fatty acid composition (g/100 g oil) in edible fats and oils.

<table>
<thead>
<tr>
<th></th>
<th>CO</th>
<th>PHCO</th>
<th>MF</th>
<th>BT</th>
<th>CO</th>
<th>PHCO</th>
<th>MF</th>
<th>BT</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-C10:0</td>
<td></td>
<td></td>
<td>7.7 ± 0.2</td>
<td></td>
<td></td>
<td></td>
<td>1.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>C10:0</td>
<td></td>
<td></td>
<td>4.2 ± 0.1</td>
<td></td>
<td></td>
<td></td>
<td>3.5 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>C12:0</td>
<td></td>
<td></td>
<td>12.2 ± 0.5</td>
<td>2.8 ± 0.1</td>
<td></td>
<td></td>
<td>20.4 ± 0.6</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>C16:0</td>
<td>4.6 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>30.3 ± 0.9</td>
<td>24.6 ± 0.9</td>
<td>0.4 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>36.0 ± 0.9</td>
<td>7.3 ± 0.1</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>1.5 ± 0.1</td>
<td>2.8 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>2.1 ± 0.1</td>
<td>4.3 ± 0.0</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.0 ± 0.1</td>
<td>11.0 ± 0.3</td>
<td>9.6 ± 0.3</td>
<td>16.5 ± 0.6</td>
<td>0.4 ± 0.1</td>
<td>7.7 ± 0.1</td>
<td>5.2 ± 0.2</td>
<td>5.5 ± 0.3</td>
</tr>
<tr>
<td>trans-C18:1</td>
<td></td>
<td>46.1 ± 1.8</td>
<td>3.0 ± 0.3</td>
<td>1.5 ± 0.1</td>
<td></td>
<td></td>
<td>50.5 ± 1.5</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>cis-9-C18:1</td>
<td>51.0 ± 1.8</td>
<td>22.0 ± 0.9</td>
<td>17.9 ± 0.6</td>
<td>40.8 ± 1.2</td>
<td>50.0 ± 1.3</td>
<td>24.2 ± 0.1</td>
<td>15.1 ± 0.5</td>
<td>64.4 ± 1.8</td>
</tr>
<tr>
<td>cis-11-C18:1</td>
<td>4.0 ± 0.1</td>
<td>2.4 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>2.4 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>trans-C18:2</td>
<td>0.2 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.9 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>cis-C18:2</td>
<td>20.2 ± 1.0</td>
<td>0.1 ± 0.0</td>
<td>1.3 ± 0.1</td>
<td>1.9 ± 0.0</td>
<td>29.6 ± 0.9</td>
<td>0.2 ± 0.0</td>
<td>1.5 ± 0.1</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>trans-C18:3</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>cis-C18:3</td>
<td>9.2 ± 0.8</td>
<td>0.1 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>12.7 ± 0.5</td>
<td>0.4 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>CLA</td>
<td></td>
<td></td>
<td>0.5 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td></td>
<td></td>
<td>0.4 ± 0.0</td>
<td>0.4 ± 0.0</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C20:1</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td></td>
<td></td>
<td></td>
<td>0.1 ± 0.0</td>
</tr>
</tbody>
</table>

Each value represents mean ± SE (n=3). Contents lower than 0.1 g/100 g oil are not reported.
CO, canola oil; PHCO, partially hydrogenated canola oil; MF, milk fat; BT, beef tallow.

et al.\(^\text{25}\), which used the structured lipids 1,3-dipalmitoyl-2-oleoyl-sn-glycerol and 1,3-dioleoyl-2-palmitoyl-rac-glycerol.

In the present study, we also determined whether the double bond configuration of unsaturated fatty acids influences the fatty acid selectivity of CALB by using structured lipids containing both cis- and trans-unsaturated fatty acids as substrates. Recoveries of more than 99 mol% were obtained for OEO and OOE samples containing either fatty acid isomer at the sn-2 position. As such, double bond configuration does not seem to affect the enzymatic reaction.

3.2 Regiospecific distribution of fatty acids in edible fats and oils

The fatty acid composition of TAG and 2-MAG in edible fats and oils is detailed in Table 2. All of the trans-positional isomers of C18:1, octadecadienoic acid (C18:2), and octadecatrienoic acid (C18:3) were described as their sums. Analysis showed that all samples, with the exception of CO, had TFA compositions of primarily trans-C18:1, with concentrations in PHCO, MF, and BT of 46.1, 3.0, and 1.5 g/100 g oil, respectively. The majority of the TFA in the 2-MAG samples also consisted of trans-C18:1, with concentrations in PHCO, MF, and BT of 50.5, 2.0, and 0.7 g/100 g oil, respectively. Although trans-C18:1 was not detected in CO (less than 0.1 g/100 g oil), CO showed very low
trans-C18:2 and trans-C18:3 concentrations, with values of 0.2 and 0.1 g/100 g oil, respectively. These compounds are formed by deodorization in the refining process of vegetable oil.

PHCO used in the present study was prepared by partial hydrogenation of CO in the presence of nickel. Therefore, we compared the fatty acid compositions of CO and PHCO to investigate the effect of partial hydrogenation on fatty acid composition. The results showed more saturated fatty acids (SFAs), such as stearic acid (C18:0), and TFA, including C18:1, C18:2, and C18:3, in PHCO than in CO. In contrast, the contents of cis-unsaturated fatty acids such as C18:1 and C18:2, and C18:3 were lower in PHCO. These results indicate that partial hydrogenation converted the cis-unsaturated fatty acids into SFAs and TFA.

In addition to trans-C18:1, ruminant fats are the only fats which contain conjugated linoleic acid (CLA), which mainly consists of rumenic acid (cis-9, trans-11-C18:2). CLA content in MF and BT were found to be 0.5 and 0.2 g/100 g oil, respectively. It has been reported that CLA is formed by biohydrogenation of PUFAs in the rumen. Overall, CLA content in milk and beef range from 0.3 to 1.0 g/100 g oil and from 0.1 to 0.7 g/100 g oil, respectively. Our results are in good agreement with these values.

The regiospecificity of each fatty acid at the sn-2 position of TAG in CO, PHCO, MF, and BT is shown in Fig. 1. CO showed even distribution of cis-9-C18:1, the most abundant fatty acid in the sample at 51.0 g/100 g oil, based on a regiospecificity of 32.7 mol/100 mol that showed insignificant variation from the expected value of 33.3 mol/100 mol (p > 0.05). In contrast, cis-C18:2 and cis-C18:3 values were significantly higher than 33.3 mol/100 mol, indicating preferential binding to the sn-2 position. These findings are consistent with previous studies.

PHCO gave selective binding of trans-C18:1, the most abundant fatty acid in the sample at 46.1 g/100 g oil, at the sn-2 position (p < 0.05), although considerable amounts of the compound were also esterified at the sn-3 (3) position. This binding tendency is similar to those of cis-C18:2 and cis-C18:3 in CO, suggesting that trans-C18:1 located at the sn-2 position is mainly formed by partial hydrogenation of cis-unsaturated fatty acids located at the same position.

The regiospecific distribution of fatty acids in ruminant TAG is consistent and well understood. For instance, in MF, C18:0 is selectively esterified at the sn-1 position, myristic acid (C14:0) is preferentially located at the sn-2 position, and SCFAs are esterified almost exclusively at the sn-3 position. Meanwhile, in BF, C16:0 tends to bind to the sn-1 position, cis-9-C18:1 is selectively located at the sn-2 position, and C18:0 is preferentially esterified at the sn-3 position. In the present study, we observed the same fatty acid distribution pattern, although our analytical method is not capable of distinguishing the difference between the sn-1 and 3 positions. Namely, in MF, SCFAs (C10:0) and C18:0 were esterified at the sn-1 (3) position, whereas C14:0 was bound to the sn-2 position. In BT, C16:0 and C18:0 were located at the sn-1 (3) position, whereas cis-9-C18:1 was esterified at the sn-2 position. As previously observed, trans-C18:1, the most dominant TFA, was preferentially bound to the sn-1 (3) position in ruminant fats (p < 0.05).

TAG for MF and ruminant depot fat is mainly biosynthesized via the glycerol-3-phosphate pathway, in which fatty acids are esterified from the sn-1 to the sn-3 positions of the glycerol backbone by several enzymes, such as glycerol-3-phosphate acyltransferase. However, these enzymes show different fatty acid selectivity. Note that phospholipid (PL) is also synthesized via this pathway. Some studies investigating fatty acid distribution for PL have reported that trans-C18:1 is selectively incorporated into the sn-1 position, suggesting that trans-C18:1 is also esterified at the sn-1 position of ruminant TAG.

We also observed that CLA in MF was bound to the sn-1 (3) position, whereas CLA in BT was bound to the sn-2 position.
position \((p<0.05)\); both of these observations are consistent with previous studies. Mir et al. found that muscle fat from beef steers has greater proportions of CLA at the sn-2 position\(^{30}\). Meanwhile, Chardigny et al. revealed that rumenic acid, the major isomer of CLA in butter and cheese, is esterified mainly in the sn-1(3) position of TAG, with 74% appearing there\(^{36}\). They also suggested that CLA bound to the sn-1(3) position is better absorbed and \(\beta\)-oxidized than CLA bound to the sn-2 position. This suggests that incorporation of CLA at the sn-1(3) position allows for easy conversion of energy, which likely contributes to the health of newborn calves.

3.3 Regiospecific distribution of trans-C18:1 positional isomers

The relative proportion of trans-C18:1 positional isomers in TAG and 2-MAG samples from PHCO, MF, and BT is detailed in Fig. 2. The majority of trans-C18:1 isomers in the PHCO TAG sample consisted of trans-9-C18:1 (21.4% of total content), followed by trans-10-C18:1 (21.3%), trans-8-C18:1 (15.3%), and trans-11-C18:1 (13.2%); similar results were obtained for the 2-MAG fraction. On the other hand, the MF TAG sample gave trans-11-C18:1 (37.2%) as the dominant isomers, with smaller amounts of trans-16-C18:1 (11.1%), trans-14-C18:1 (10.0%), trans-10-C18:1 (8.5%), and trans-13-C18:1 (8.2%). Likewise, the major isomers in the BT TAG sample were found to be trans-11-C18:1 (31.5%) and trans-10-C18:1 (22.8%), which together accounted for more than half of all positional isomers. Interestingly, the relative proportion of trans-10-C18:1 in the 2-MAG fractions were higher than for TAG, with MF and BT proportions of 12.6 and 35.7%, respectively. Notably, trans-10-C18:1 replaced trans-11-C18:1 as the most abundant isomer in BT.

In the present study, we observed regiospecific distribution of individual trans-C18:1 positional isomers in TAG; the specificity for the sn-2 position in PHCO, MF, and BT is shown in Fig. 3. Values of trans-9-C18:1 and trans-11-C18:1, the most abundant TFA isomers, were significantly higher than 33.3 mol/100 mol in PHCO; the same applies to a lesser extent for all other trans-C18:1 positional isomers, except for trans-4-C18:1. These data indicate that all major trans-C18:1 positional isomers are selectively located at the sn-2 position of TAG in PHCO. However, most trans-C18:1 positional isomers in ruminant fats were preferentially located at the sn-1(3) position, as demonstrated by the fact that regiospecificity for all compounds, except for trans-7-C18:1 and trans-8-C18:1, were lower than 33.3 mol/100 mol at the sn-2 position. Meanwhile, comparing trans-11-C18:1 and trans-10-C18:1, the most abundant TFAs in ruminant fats, showed that trans-11-C18:1 bound to the sn-1(3) position more selectively \((p<0.05)\).

In addition, trans-C18:1 isomers with double bonds at odd positions tended to be located at sn-1(3) positions more than those with double bonds at even positions, sug-

![Fig. 2](image1.png)

**Fig. 2** Relative proportion of trans-octadecenoic acid (trans-C18:1) positional isomers (wt% of total trans-C18:1) in (a) TAG and (b) 2-MAG for PHCO, MF, and BT.

![Fig. 3](image2.png)

**Fig. 3** Regiospecificity of trans-octadecenoic acid (C18:1) positional isomers at the sn-2 position of TAG (mean ± SE) in PHCO, MF, and BT. The dotted line at 33.3 mol/100 mol indicates the regiospecific value at which the fatty acid is equally distributed. *Significantly different from 33.3 mol/100 mol \((p<0.05)\).
gesting that individual isomers may be distinguishable in vivo. This hypothesis is supported by previous research. Specifically, Lawson & Holman observed that most even-positioned trans-C18:1 isomers undergo β-oxidation more rapidly in liver mitochondria⁷, while Gunstone demonstrated that the melting points of even-positioned trans-C18:1 isomers are higher. As such, the different treatment of these compounds likely results from differences in metabolic and physical properties⁸.

In summary, we tested sn-1(3) selective transesterification with CALB using structured lipids, and revealed that differences in fatty acid double bond configuration do not affect enzymatic activity. Next, we investigated the regiospecific distribution of TFA in CO, PHCO, MF, and BT; trans-9-C18:1 was located at the sn-2 position of PHCO TAG, while most trans-C18:1 was found at the sn-1(3) position of TAG for ruminant fats. Previous studies have reported that the absorption and function of fatty acids depend on their binding position on TAG, suggesting that the metabolic activity of TFA might also be dependent on this factor. Additional studies taking the binding position of TFA on TAG into account are needed to clarify their metabolic behavior and identify TFA isomers that contribute the most to CVD.

REFERENCES