Resolution Behavior of *cis*- and *trans*-Octadecenoic Acid Isomers by AOCS Official Method Using SP-2560 Column

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Abstract: The gas chromatography–flame ionization detector equipped with a higher polarity column (i.e., SP-2560) has often been used for the quantification of *trans*-fatty acids in food. In particular, AOCS Ce 1h-05, the official method of the American Oil Chemists' Society (AOCS), is a highly effective method to separate the isomers of *trans*-fatty acids. In this study, the resolution behavior and the response factors of *cis*- and *trans*-octadecenoic acid methyl ester (C18:1-ME) isomers separated by the AOCS Ce 1h-05 method were investigated, and the contents of each *cis*- and *trans*-C18:1-ME isomer in partially hydrogenated vegetable oil (PHVO) and milk fat were quantified by using the calibration curves obtained for the respective isomers. The relative response factors for the *trans*- and *cis*-C18:1-ME isomers against the internal standard heneicosanoic acid methyl ester (C21:0-ME) were 1.031 ± 0.040 (mean $\pm$ SD) and 0.990 ± 0.032 , respectively. The relative response factors of *trans*-isomers tend to be higher than those of *cis*-C18:1-ME isomers. The peaks of *cis*-4-C18:1-ME, *cis*-5-C18:1-ME, *cis*-6-C18:1-ME, *cis*-7-C18:1-ME, *cis*-8-C18:1-ME, and *cis*-9-C18:1-ME isomers overlapped with those of *trans*-C18:1-ME isomers. Both PHVO and milk fat contained many types of *cis*- and *trans*-C18:1 isomers, and the total contents of the *trans*-C18:1 isomer in PHVO and milk fat were 28.01 g and 3.62 g per 100 g oil, respectively. When the *trans*-C18:1-ME isomer was separated from the *cis*-C18:1-ME by using a silver-ion cartridge column before the analyses, the total contents of the *trans*-C18:1 isomer in PHVO and milk fat were 23.03 g and 2.78 g per 100 g oil, respectively. The difference in the *trans*-C18:1 isomer content between the two methods was ascribed to the partial overlapping of *cis*-isomer peaks with the peaks of *trans*-C18:1-ME isomers, in the chromatogram.

Key words: AOCS official method, GC-FID, Resolution, *Trans*-fatty acid isomer

1 INTRODUCTION

Trans-fatty acids are the geometrical isomers of mono-unsaturated and polyunsaturated fatty acids having non-conjugated, interrupted by at least one methylene group, carbon-carbon double bonds in the *trans* configuration¹. The primary *trans*-fatty acid isomers in foods consist of 13 octadecenoic acid (C18:1) isomers with double-bond positions from $\Delta 4$ to $\Delta 16$ ². The two major ways in which dietary *trans*-fatty acids are formed are partial hydrogenation of vegetable oil and bacterial isomerization in the rumen of ruminants³. In particular, partially hydrogenated vegetable oil (PHVO) characteristically contains a high percentage of elaidic acid (*trans*-9-C18:1 or *trans*-C18:1n-9)².

In contrast, *trans*-fatty acids in ruminant fats have a high percentage of vaccenic acid (*trans*-11-C18:1 or *trans*-C18:1n-7)⁴.

Epidemiological studies have shown that high dietary intake of PHVO increases low-density lipoprotein (LDL)

Abbreviations: C18:1, octadecenoic acid; C21:0-ME, heneicosanoic acid methyl ester; C21:0-TAG, triheneicosahexaen; GC-FID, gas chromatography–flame ionization detector; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LOD, limit of detection; LOQ, limit of quantification; ME, methyl ester; PHVO, partially hydrogenated vegetable oil; R_f , response factor; R_s , resolution factor; s/n , signal-to-noise ratio; *trans*-9-C18:1, elaidic acid; *trans*-11-C18:1, vaccenic acid

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and decreases high-density lipoprotein (HDL) concentration in blood^{5,6)}, and consequently, this results in adult diseases. On the other hand, many studies prove that the intake of ruminant *trans*-fatty acids has no relationship to coronary heart disease, and there is still controversy concerning the difference in the effects of *trans*-fatty acid species on health. Furthermore, the different health effects are also pointed out in the Risk Profile Sheet published by the Ministry of Agriculture, Forestry and Fisheries in Japan⁷⁾. Therefore, it is very important to distinguish between different *trans*-fatty acid isomers in foods. However, no method is available to completely quantify the amount of the respective isomers for the main *trans*-fatty acids in foods.

The *trans*-fatty acids in foods are identified using a gas chromatography-flame ionization detector (GC-FID), equipped with the high-polarity column (SP-2560 column). There are several official methods such as the AOCS official method "Ce 1h-05"⁸⁾, AOAC (Association of Official Analytical Chemists) International official method "996.06"⁹⁾, and JOCS (Japan Oil Chemists' Society) official method "2.4.4.3-2013"¹⁰⁾. Although these methods can partially separate *cis*- and *trans*-C18:1 methyl ester (C18:1-ME) isomers, several peaks of *cis*- and *trans*-C18:1-ME isomers overlap on the chromatogram. Therefore, it is difficult to quantify a precise amount of *cis*- and *trans*-C18:1-ME isomers in the foods. To overcome these problems, several different approaches have been used to analyze the *cis*- and *trans*-C18:1-ME isomers. To provide a definitive composition of fatty acids, a prior separation of *cis*- and *trans*-C18:1-ME isomers by silver-ion thin layer chromatography (Ag-TLC) and silver-ion high-performance liquid chromatography (Ag-HPLC) has been employed^{11, 12)}. However, this procedure is time-consuming. Recently, Goto *et al.* reported a simple pre-fractionation method by using silver-ion solid-phase extraction (Ag-SPE)¹³⁾. As an alternative approach without prior silver-ion fractionation, Kramer *et al.* separated *cis*- and *trans*-C18:1-ME isomers using different GC temperature programs. This method takes advantage of differences in the relative retention time between *cis*- and *trans*-C18:1-ME isomers, depending on the GC temperature¹⁴⁾. However, this tendency depends on the type of columns. Consequently, the risk of misidentification of *cis*- and *trans*-C18:1-ME isomers exists when using this method.

The AOCS official method, Ce 1h-05, is one of the conventional methods to analyze *cis*- and *trans*-C18:1-ME isomers. However, the resolution characteristics such as the resolution and response factor, elution order at various temperatures, and overlapping components of *cis*- and *trans*-C18:1-ME isomers have not yet been properly investigated. Moreover, there is not enough information on whether the contents of *trans*-fatty acids in foods are affected by prior separation of *cis*-fatty acids.

In this study, the resolution behavior and the resolution and response factors for determination of *cis*- and *trans*-C18:1-ME isomers were assessed by analysis of reference standards of respective *cis*- and *trans*-C18:1-ME isomers with the Ce 1h-05 method. To investigate the effect of overlapping of the peaks of *cis*- and *trans*-fatty acids, the contents of *cis*- and *trans*-C18:1-ME isomers in PHVO and milk fat were quantified by the Ce 1h-05 method and compared with those obtained by using Ag-SPE.

2 EXPERIMENTAL

2.1 Chemicals and materials

All the *cis*- and *trans*-C18:1-ME isomers used as reference materials are shown in Fig. 1 (purity: >99%). These C18:1-ME isomers, heneicosanoic acid methyl ester (C21:0-ME) and triheneicosanoin (C21:0-TAG), and other reagents were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). PHVO and butter were in-house products (Tsukishima Foods Industry Co. Ltd., Tokyo, Japan). Partially hydrogenated rapeseed oil having an iodine value of 71.5 was selected as a PHVO.

2.2 Analytical conditions

One microliter of the C18:1-ME isomer in the hexane solution was subjected to GC-FID (TRACE GC ULTRA, Thermo Fisher Scientific Inc., Waltham, MA) equipped with a cyanopropyl capillary column (SP-2560, 100 m × 0.25 mm ID, 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 carrier gas (helium) was 1.0 mL/min.

2.3 Resolution characteristics of *cis*- and *trans*-C18:1-ME isomers

2.3.1 Calibration curves for C18:1-ME isomers

The calibration curves for the respective C18:1-ME isomers, limit of quantification (LOQ), and limit of detection (LOD) by the Ce 1h-05 method were investigated. The calibration curves were obtained by plotting the concentration ratio (C18:1-ME/C21:0-ME) on the *x*-axis and the chromatogram peak area ratio (C18:1-ME/C21:0-ME) on the *y*-axis for the respective *cis*- and *trans*-C18:1-ME isomers. The detection and quantification limit of this method were calculated by using the signal-to-noise ratio (*s/n*). The values of LOD and LOQ were defined as *s/n* = 3 and 10, respectively¹⁵⁾.

2.3.2 Resolution and relative response factor

The resolution factors for the two C18:1-ME isomer peaks adjoining each other and the response factor of *cis*- or *trans*-C18:1-ME isomers were examined by using a standard solution prepared by dissolving 13 types of *cis*- or

Fig. 1 Structures of fatty acid methyl esters used in this study.

trans-C18:1-ME isomers in hexane. The standard solution also contained the C21:0-ME isomer as the internal standard.

The resolution factor (R_s) was calculated from the following equation¹⁶⁾:

$$R_s = 2(t_{R2} - t_{R1}) / (W_1 + W_2),$$

where t_{R1} and t_{R2} are retention times, and W_1 and W_2 are the widths of the first- and second-eluted peaks, respectively.

The relative response factor (R_F) was calculated from the following equation:

$$R_F = P_s C_a / P_a C_s,$$

where P_a and P_s are the peak areas and C_a and C_s are the concentrations of analyte and internal standard, respectively.

2.3.3 Elution order of *cis*- and *trans*-C18:1-ME isomers at various column temperatures

The effect of column temperature on the elution order of the respective *cis*- and *trans*-C18:1-ME isomers was investigated. The standard sample solutions were prepared by dissolving 13 types of *trans*-C18:1-ME isomers in hexane to obtain a final concentration of 100 μ g/mL for the respective fatty acids. The standard solution of *cis*-C18:1-ME isomer was also prepared in the same way. At five different

column temperatures – 170, 175, 180, 185, and 190°C – the elution orders were investigated and compared under these conditions.

2.4 Analysis of edible fats and oils

2.4.1 Extraction of lipids and its methyl esterification

Milk fat was extracted from butter by the Folch procedure¹⁷⁾. The extracted milk fat or PHVO was methyl esterified according to the AOCS official method, Ce 1b-89¹⁸⁾. 100 mg of this sample was mixed with 1 mL of a 5 mg/mL solution of C21:0-TAG in chloroform in a screw-capped test tube. After the solvent was evaporated, the mixture was added to 1.5 mL of 0.5 N sodium hydroxide in methanol and heated at 100°C for 9 min. The resulting solution was added to 2 mL of 14% solution of boron trifluoride in methanol and reheated at 100°C for 9 min. After cooling the hot solution to room temperature, 1 mL of hexane and 5 mL of saturated saline solution were added to it and mixed vigorously. The upper hexane layer was moved to another screw-capped sample tube and used as the test sample solution after dehydrating with anhydrous sodium sulfate.

2.4.2 Resolution of *cis*- and *trans*-18:1-ME using silver-ion cartridge column

The resolution of *cis*- and *trans*-C18:1-ME isomers was carried out by using a silver-ion SPE cartridge¹³⁾ (Ag-ION

SPE, Discovery Ag-ION, Sigma-Aldrich Japan K. K., Tokyo, Japan). The silver-ion cartridge column was conditioned by passing 4 mL of acetone followed by 8 mL of hexane. 0.5 mg of the sample was loaded into the cartridge, and 6 mL of dichloromethane was passed to remove the saturated fatty acid methyl ester. Then, the *trans*-C18:1-ME isomer was collected by using 6 mL of the dichloromethane and ethyl acetate mixture (90/10, v/v). Finally, the *cis*-C18:1-ME isomer was collected by using 6 mL of the dichloromethane and ethyl acetate mixture (75/25, v/v).

2.4.3 Quantification of *cis*- and *trans*-C18:1 isomers in edible fats and oils

The *cis*- and *trans*-fatty acid isomers in PHVO and milk fat were analyzed by using the AOCS Ce 1h-05 method (Section 2.2) with or without separating the *cis*- and *trans*-C18:1 isomers before the analysis and by using a silver-ion cartridge column. Identification of the respective fatty acid isomers was carried out by using the retention times of the standard samples. The contents of respective fatty acids were adjusted by using the internal standard peak area and were calculated as grams per 100 g oil.

3 RESULTS

3.1 Resolution of *cis*- and *trans*-C18:1-ME isomers by Ce 1h-05

The calibration curve of the *trans*-9-C18:1-ME isomer was plotted between 10 and 1000 µg/mL and was expressed as a first-order equation ($R^2 > 0.999$). The values of LOD and LOQ were 0.40 and 0.11 µg/mL, respectively. Other calibration curves for the C18:1-ME isomers (Fig. 1)

were also constructed, all of which were expressed as a first-order equation. Their LOD and LOQ values were almost the same as those of the *trans*-9-C18:1-ME isomers.

The R_F values were calculated against C21:0-ME as the internal standard. For *trans*-C18:1-ME and *cis*-C18:1-ME samples, they were 1.031 ± 0.040 (mean $\pm$ SD) and 0.990 ± 0.032 , respectively. The R_F values for *trans*-isomers tend to be higher than those of *cis*-C18:1-ME isomers (Fig. 2). Although the values of R_F were different, the relative standard deviations for all *cis*- and *trans*-C18:1-ME isomers were $< 5\%$. The theoretical R_F values, which have been used in Ce 1h-05, is 1.0155 (dotted line in Fig. 2), which was located within the range of R_F distribution. Moreover, the average R_F for both the *cis*- and *trans*-C18:1-ME isomers obtained was 1.010 ± 0.040 , which is approximately 1.0155.

The elution order of *trans*-C18:1-ME isomers was as follows: the *trans*-4-C18:1-ME isomer eluted first, while the *trans*-16-C18:1-ME isomer eluted last (Fig. 3). However, the *trans*-6-C18:1-ME, *trans*-7-C18:1-ME, and *trans*-8-C18:1-ME isomers were eluted together. In addition, the peaks of *trans*-13-C18:1-ME and *trans*-14-C18:1-ME isomers were resolved at the same time. In the case of *cis*-C18:1-ME isomers, it was found that the *cis*-7-C18:1-ME eluted ahead of the *cis*-6-C18:1-ME isomer (data not shown). However, a good resolution could not be achieved for *cis*-6-C18:1-ME, *cis*-7-C18:1-ME, and *cis*-8-C18:1-ME isomers in the standard sample mixture. In addition, *cis*-4-C18:1-ME and *cis*-5-C18:1-ME isomers were eluted together. The R_s values for *trans*- and *cis*-C18:1-ME isomers are shown in Tables 1 and 2, respectively. These results indicated that the pairs of *trans*- and *cis*-C18:1-ME isomers

Fig. 2 Response factors of fatty acid methyl ester samples. The dotted line (1.0155) indicates the theoretical value obtained using the AOCS official method Ce 1h-05 (mean $\pm$ SD, $n = 3$).

Fig. 3 GC-FID chromatograms of *trans*- and *cis*-fatty acid methyl ester samples eluted at 180°C.

Table 1 Resolution factors of *trans*-C18:1-ME isomers.

Double bond position	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16
Resolution factor	1.91	1.19	0.14	0.09	0.65	0.58	0.54	1.19	0.71	0.11	1.61	6.75

Table 2 Resolution factors of *cis*-C18:1-ME isomers.

Double bond position	4-5	5-7	7-6	6-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16
Resolution factor	0.30	3.21	0.29	0.30	0.89	0.87	1.22	1.72	1.86	1.89	1.78	10.41

having an R_s value of <0.30 could not be separated on the chromatogram. The R_s value between *trans*-6-C18:1-ME and *trans*-11-C18:1-ME isomers was <1.0 , thus, a complete resolution was not accomplished under the present analytical conditions.

3.2 Elution order of *cis*- and *trans*-C18:1-ME isomers at various column temperatures

The elution orders of *trans*- and *cis*-C18:1-ME isomers at 170–190°C are shown in **Table 3**. The peaks for the following groups of isomers overlapped on the chromatogram at 180°C: “*cis*-4-C18:1-ME, *cis*-5-C18:1-ME, and *trans*-9-C18:1-ME isomers,” “*cis*-6-C18:1-ME, *cis*-7-C18:1-ME, *cis*-8-C18:1-ME, *trans*-13-C18:1-ME, and *trans*-14-C18:1-ME isomers,” and “*cis*-9-C18:1-ME and *trans*-15-C18:1-ME isomers.” Some of the elution orders reversed at the five different column temperatures; this indicates that co-eluting components (dotted frame in **Table 3**) are changed by GC temperature. The resolution conditions for

the *trans*- and *cis*-C18:1-ME isomers were improved by lowering the column temperature; however, instead of improving the resolution, the elution times corresponding to *trans*- and *cis*-C18:1-ME isomer peaks gradually became closer.

3.3 Contents of *cis*- and *trans*-C18:1 isomers in edible fats and oils

The contents of the respective fatty acids in PHVO and milk fat, which are determined without the silver-ion cartridge column used for the separation of the *cis*- and *trans*-C18:1 isomers, are shown in **Table 4**. All the inseparable peaks of *trans*-9-C18:1-ME, *cis*-4-C18:1-ME, and *cis*-5-C18:1-ME isomers were considered as *trans*-fatty acids. Similarly, all the inseparable peaks of *trans*-13-C18:1-ME, *trans*-14-C18:1-ME, *cis*-6-C18:1-ME, *cis*-7-C18:1-ME, and *cis*-8-C18:1-ME isomers were also considered as *trans*-fatty acids. In contrast, inseparable peaks of *trans*-15-C18:1-ME and *cis*-9-C18:1-ME isomers were considered as *cis*-

Table 3 Elution orders of respective C18:1-ME isomers at 5 kinds of column temperatures.

170°C	175°C	180°C	185°C	190°C	Elution order ↓ Earlier Later
<i>t</i> -4	<i>t</i> -4	<i>t</i> -4	<i>t</i> -4	<i>t</i> -4	
<i>t</i> -5	<i>t</i> -5	<i>t</i> -5	<i>t</i> -5	<i>t</i> -5	
<i>t</i> -6,7,8 <i>c</i> -4,5	<i>t</i> -6,7,8 <i>c</i> -4,5	<i>t</i> -6,7,8 <i>c</i> -4,5	<i>t</i> -6,7,8 <i>t</i> -9 <i>c</i> -4,5	<i>t</i> -6,7,8 <i>t</i> -9 <i>c</i> -4,5	
<i>t</i> -9	<i>t</i> -9	<i>t</i> -9	<i>t</i> -10	<i>t</i> -10 <i>t</i> -11	
<i>t</i> -10	<i>t</i> -10	<i>t</i> -10	<i>t</i> -11	<i>t</i> -12	
<i>t</i> -11	<i>t</i> -11	<i>t</i> -11	<i>t</i> -12	<i>t</i> -13,14 <i>c</i> -6,7,8	
<i>t</i> -12	<i>t</i> -12	<i>t</i> -12	<i>t</i> -13,14 <i>c</i> -6,7,8	<i>t</i> -15	
<i>c</i> -6,7,8 <i>t</i> -13,14	<i>c</i> -6,7,8 <i>t</i> -13,14	<i>t</i> -13,14 <i>c</i> -6,7,8	<i>t</i> -15 <i>c</i> -9	<i>c</i> -9	
<i>c</i> -9 <i>c</i> -10	<i>c</i> -9 <i>t</i> -15	<i>c</i> -9 <i>t</i> -15	<i>c</i> -10	<i>c</i> -10	
<i>t</i> -15	<i>c</i> -10	<i>c</i> -10	<i>c</i> -11	<i>c</i> -11	
<i>c</i> -11	<i>c</i> -11	<i>c</i> -11	<i>c</i> -12	<i>c</i> -12	
<i>c</i> -12	<i>c</i> -12	<i>c</i> -12	<i>c</i> -13	<i>c</i> -13	
<i>c</i> -13	<i>c</i> -13	<i>c</i> -13	<i>t</i> -16 <i>c</i> -14	<i>t</i> -16 <i>c</i> -14	
<i>t</i> -16 <i>c</i> -14	<i>t</i> -16 <i>c</i> -14	<i>t</i> -16 <i>c</i> -14	<i>c</i> -15	<i>c</i> -15	
<i>c</i> -15	<i>c</i> -15	<i>c</i> -15	<i>c</i> -16	<i>c</i> -16	

trans : *t*, *cis* : *c*

fatty acids.

Both PHVO and milk fat contained many types of *cis*- and *trans*-C18:1 isomers, and the total contents of *trans*-C18:1 in PHVO and milk fat were 28.01 g and 3.62 g per 100 g oil, respectively, when the *cis*- and *trans*-C18:1 isomers were not separated by using a silver-ion cartridge column. When the *cis*- and *trans*-C18:1 isomers were separated before the analyses, the total contents of the *trans*-C18:1 isomer in PHVO and milk fat were 23.03 g and 2.78 g per 100 g oil, respectively.

4 DISCUSSION

4.1 Resolution character of *cis*- and *trans*-C18:1-ME isomers by Ce 1h-05

The GC-FID performance for the quantification of *cis*- and *trans*-C18:1-ME isomers includes a broad range of linear response, low LOD, and LOQ. On the basis of these results, it is concluded that the sample solution adjusted to 1% for GC analysis is acceptable for analyzing 0.01% of *cis*- and *trans*-C18:1-ME isomers because the LOQ value for all the isomers studied was 0.40 µg/mL.

The response of the FID instrument has been known to be linear, and the response behavior of the analyte depends on the number of carbon atoms and double bonds in the fatty acids. Therefore, the theoretical R_F values using Ce

1h-05 can be calculated from the structures of the respective fatty acids. However, the differences in the double-bond positions in the fatty acids and their corresponding volatility could affect their GC-FID chromatogram peak areas. In fact, the individual R_F values of *cis*- and *trans*-C18:1-ME isomers were slightly different, although the average R_F value for both the *cis*- and *trans*-C18:1-ME isomers was nearly the same as that obtained by employing AOCS method.

4.2 Elution order of *cis*- and *trans*-C18:1-ME isomers at various column temperatures

The elution order of *trans*- and *cis*-C18:1-ME isomers under AOCS Ce 1h-05 conditions (Fig. 3) are similar to the findings of a previous report^{2, 19, 20}. These orders are affected by the GC temperature. In particular, the relative retention time of *cis*-fatty acids is more affected than that of *trans*-fatty acids is. According to a previous report, the polarity of the cyanopropyl column is strongly affected by the temperature changes²¹. Furthermore, it is reported that compared to the *trans*-isomers, the *cis*-isomers were retained more strongly on the cyanopropyl stationary phase of the SP-2560 column when the column temperature was increased²².

In this study, overlapping of the peaks of some of *trans*-C18:1-ME isomers and *cis*-C18:1-ME isomers was observed (Table 3). This could lead to miscalculation of the total

Table 4 Contents of *cis* and *trans*-C18:1 in PHVO and milk fat (g/100g oil).

	PHVO (mean $\pm$ SD, n=3)			Milk fat (mean $\pm$ SD, n=3)		
	Without Ag	With Ag		Without Ag	With Ag	
	Total	<i>trans</i>	<i>cis</i>	Total	<i>trans</i>	<i>cis</i>
<i>t</i> -4-C18:1	0.12 $\pm$ 0.00	0.13 $\pm$ 0.00		0.01 $\pm$ 0.01	0.01 $\pm$ 0.00	
<i>t</i> -5-C18:1	0.23 $\pm$ 0.00	0.23 $\pm$ 0.00		0.01 $\pm$ 0.00	0.01 $\pm$ 0.00	
<i>t</i> -6,7,8-C18:1	4.09 $\pm$ 0.01	3.75 $\pm$ 0.01		0.14 $\pm$ 0.01	0.08 $\pm$ 0.00	
<i>c</i> -4,5-C18:1			0.21 $\pm$ 0.00			0.18 $\pm$ 0.03
<i>t</i> -9-C18:1	5.34 $\pm$ 0.13	4.42 $\pm$ 0.01		0.19 $\pm$ 0.02	0.08 $\pm$ 0.00	
<i>t</i> -10-C18:1	5.13 $\pm$ 0.15	3.92 $\pm$ 0.00		0.15 $\pm$ 0.01	0.07 $\pm$ 0.00	
<i>t</i> -11-C18:1	5.05 $\pm$ 0.08	3.43 $\pm$ 0.00		1.81 $\pm$ 0.05	1.23 $\pm$ 0.00	
<i>t</i> -12-C18:1	2.62 $\pm$ 0.02	3.14 $\pm$ 0.01		0.20 $\pm$ 0.01	0.19 $\pm$ 0.00	
<i>t</i> -13,14-C18:1		2.52 $\pm$ 0.00			0.56 $\pm$ 0.00	
<i>c</i> -6,7,8-C18:1	4.62 $\pm$ 0.01		2.44 $\pm$ 0.06	0.74 $\pm$ 0.00		0.24 $\pm$ 0.03
<i>c</i> -9-C18:1			24.27 $\pm$ 0.25			19.23 $\pm$ 0.13
<i>t</i> -15-C18:1	15.92 $\pm$ 0.01	0.68 $\pm$ 0.00		21.09 $\pm$ 0.03	0.19 $\pm$ 0.00	
<i>c</i> -10-C18:1	1.11 $\pm$ 0.01		1.53 $\pm$ 0.00	0.00 $\pm$ 0.00		0.00 $\pm$ 0.00
<i>c</i> -11-C18:1	2.04 $\pm$ 0.01		2.29 $\pm$ 0.03	0.48 $\pm$ 0.01		0.43 $\pm$ 0.02
<i>c</i> -12-C18:1	3.69 $\pm$ 0.00		3.69 $\pm$ 0.00	0.18 $\pm$ 0.01		0.18 $\pm$ 0.00
<i>c</i> -13-C18:1	0.76 $\pm$ 0.00		0.69 $\pm$ 0.01	0.07 $\pm$ 0.01		0.07 $\pm$ 0.00
<i>t</i> -16-C18:1	0.80 $\pm$ 0.01	0.80 $\pm$ 0.00		0.36 $\pm$ 0.00	0.36 $\pm$ 0.00	
<i>c</i> -14-C18:1	0.46 $\pm$ 0.00		0.48 $\pm$ 0.01	0.00 $\pm$ 0.01		0.02 $\pm$ 0.01
<i>c</i> -15-C18:1	0.47 $\pm$ 0.01		0.96 $\pm$ 0.03	0.12 $\pm$ 0.01		0.08 $\pm$ 0.00
<i>c</i> -16-C18:1	0.54 $\pm$ 0.00		0.86 $\pm$ 0.00	0.08 $\pm$ 0.01		0.07 $\pm$ 0.00
Σ <i>trans</i> -C18:1	28.01	23.03		3.62	2.78	

trans; *t*, *cis*; *c*

Without Ag: Ag cartridge column was not used for the separation of *cis*- and *trans*-fatty acids before analysis

With Ag: Ag cartridge column was used for the separation of *cis*- and *trans*-fatty acids before analysis

content of *trans*-fatty acids in foods. In fact, the peak corresponding to elaidic acid, a *trans*-9-C18:1 isomer, which is the dominant *trans*-fatty acid species in PHVO (occupied 15.2-46.1% of *trans*-fatty acid²⁾), overlapped with several peaks on the chromatogram.

4.3 Contents of *cis*- and *trans*-C18:1 isomers in edible fats and oils

The total contents of *trans*-C18:1 in PHVO and milk fat were higher than the results of Ag-SPE analysis (28.01 g and 3.62 g per 100 g oil versus 23.03 g and 2.78 g per 100 g oil). It is concluded that the differences in the contents between the two methods could be ascribed to the overlapping of some of the *cis*-C18:1-ME isomer peaks with the peaks of the *trans*-C18:1-ME isomers. For example, the fatty acid content of *cis*-4-C18:1 and *cis*-5-C18:1 isomers was 0.21 g per 100 g oil, while that of *cis*-6-C18:1, *cis*-7-C18:1, and *cis*-8-C18:1 isomers was 2.44 g per 100 g oil in PHVO (Table 4). Previous studies reported that minor *cis*-

C18:1 isomers ranging from *cis*-6 to *cis*-15 except *cis*-9 exist in PHVO and milk fat^{4, 14, 19)}. The *cis*-C18:1 and *trans*-C18:1 isomer peaks overlapped. This could lead to miscalculation of the total *trans*-fatty acid content. Lowering the column temperature could improve the resolution of *cis*- or *trans*-C18:1-ME isomer peaks; however, the overlapping of those peaks could lead to adverse results.

The resolution behavior and R_F values of the *cis*- and *trans*-C18:1-ME isomers were examined in this study. The results showed that the R_F values for the C21:0-ME, *cis*-C18:1-ME, and *trans*-C18:1-ME isomers showed little difference among them; however, the respective values were near the theoretical value of 1.0155, as indicated in the Ce 1h-05 method. Consequently, the Ce 1h-05 method that has been used in this study for the quantification of *trans*-fatty acid content in food was found reasonable even if all the *trans*-C18:1 peaks were adapted to 1.0155 for the calculation. In contrast, the resolution of *cis*- and *trans*-

C18:1-ME isomers did not improve on changing the column temperature. The development of a method for quantifying the respective *cis*- and *trans*-C18:1-ME isomers is extremely important for revealing their effect on the lipid metabolism. Development of new methods for their resolution might be possible using higher-polarity GC columns, which is our next research target.

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