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Application Note

No.28

Trans Fatty Acid Analysis by FTIR and GC/FID, and Simple Pretreatment of Food Samples

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Food

1. Introduction

Analysis of trans fatty acids in processed foods is mainly conducted by gas chromatography (GC) using a high-resolution capillary column and a flame ionization detector (FID), or by GC with a mass spectrometer (MS). Separation of fatty acid methyl ester (FAME) isomers, particularly cis- and trans- isomers, is often conducted using a high-polarity cyanopropyl capillary column. However, when using a high-polarity column to analyze processed foods which typically contain a variety of contaminants, quantitation can be difficult due to incomplete separation of the many isomers present, even though separation can take as long as an hour per sample. On the other hand, measurement of fats and oils by FTIR can be conducted directly, taking only about 5 minutes including sample placement and rinsing. As effective as this may seem however, here too, there is a concern regarding contaminants in the food product.

Up to now, not only has the extraction of fats and oils from food and biological samples had a large impact on the environment due to the heavy use of such solvents as chloroform and methanol, the methylation operation required for GC/FID and GC/MS analysis was also cumbersome.

Here, under the guidance of Mr. Kouhei Yamamoto of Osaka Prefecture University, processed foods were subjected to saponification treatment and conventional solvent extraction, and the obtained sample extracts were analyzed by both FTIR and GC/FID methods. Here we report on our investigation comparing the differences in the trans fatty acid methyl ester quantitative results due to the respective analytical sample preparation and analysis methods.

1 Analytical & Measuring Instruments Division

2. Sample Pretreatment Using Saponification Treatment

To each of the processed food samples, 300 g of water was added to 300 g of sample, and after homogenizing, 20 g was transferred to a 100 mL high-pressure decomposition vessel. Heptadecanoic acid (17:0 FA, margaric acid) was added as an internal standard at an amount corresponding to the total fats and oils in the food. After adding 5 mL of 50 % aqueous potassium hydroxide and mixing, and then adding 20 mL ethanol and mixing well, saponification was conducted by heating at 105 °C for 30 minutes in an autoclave. The non-saponifiable substances were then extracted and removed from the saponified sample by conducting 3 washings using 30 mL hexane per washing. After adding 10 mL hydrochloric acid to acidify, the sample was heated at 105 °C for 10 minutes, followed by 3 washings with 30 mL hexane per washing to recover the fatty acids. Methylation was conducted by adding 1.5 mL of 14 % boron trifluoride/methanol solution to the recovered fatty acids, and the mixture was heated at 98 °C for 5 minutes. The obtained methylated fatty acids were purified as necessary using a silica gel column (Fig. 2-1).
20 g of the same homogenized sample was weighed out, extraction was conducted using the conventional chloroform/methanol extraction method, and this was then compared with the methylated product (Fig. 2-2).

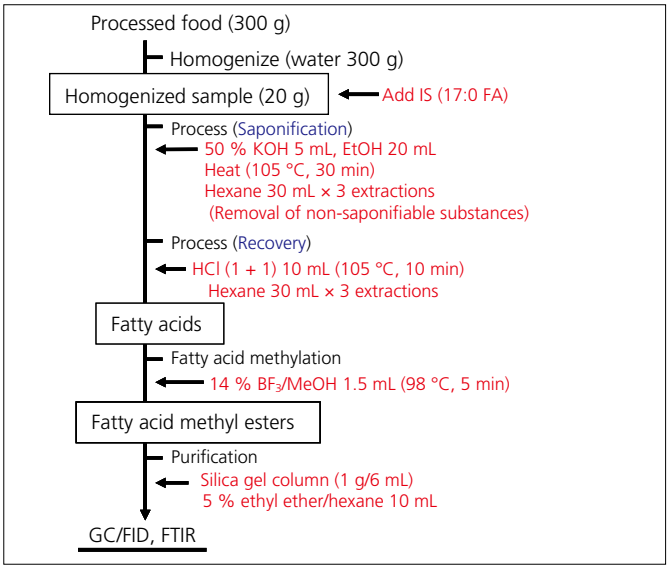

Fig. 2-1 Saponification Treatment Flow

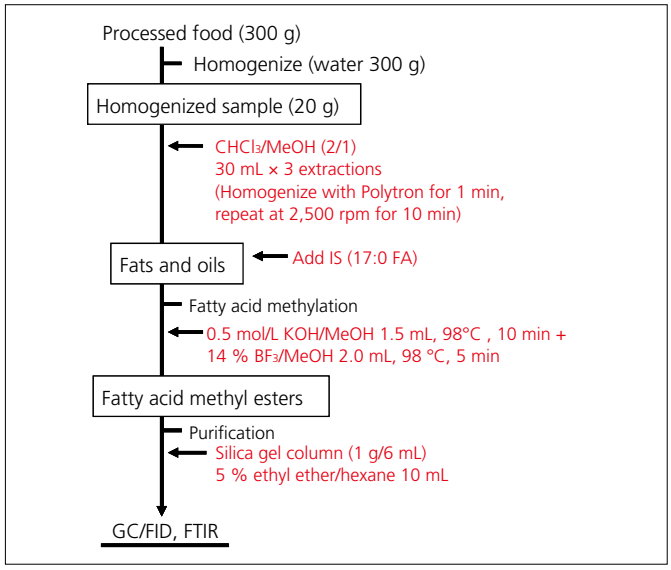

Fig. 2-2 Solvent Extraction Flow

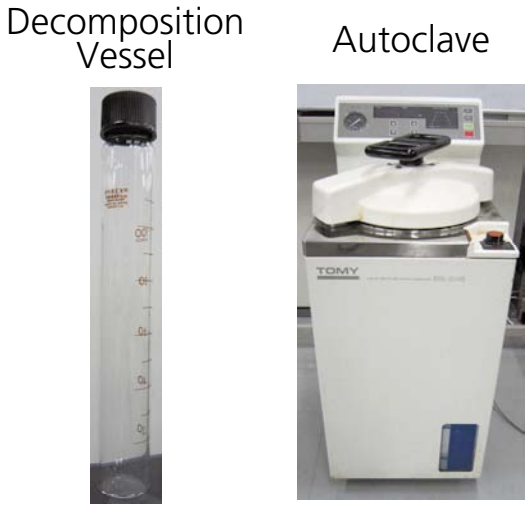

Fig. 2-3 Decomposition Vessel and Autoclave

To ensure good recovery with normal saponification, it should be verified beforehand that there is no leakage from the cap of the high-pressure decomposition vessel even under high pressure. In addition, care should be taken to ensure that there are no cuts or deterioration of the fluoropolymer screw cap seal, because contaminants originating from the seal could contaminate the sample and generate interference peaks in the GC chromatogram.

3. Analysis by FTIR

Compared to GC, use of FTIR for separation and quantitative analysis of the various trans fatty acids is difficult due to the different chain lengths and different numbers and locations of double bonds. However, because trans unsaturated fatty acids display an infrared absorption peak in the vicinity of 966 cm^{-1}, where none is displayed for the cis isomer, it is possible to conduct quantitative analysis of trans fatty acids based on the intensity of this peak. Furthermore, since measurement by FTIR of a single sample takes just seconds, many samples can be analyzed in a short period of time.

However, besides fats and oils, processed foods also contain a mixture of large amounts of other substances, including water, proteins and sugars, etc., preventing the direct analysis of foods other than fat and oil products. On the other hand, pretreatment operations such as extraction and methylation are absolutely required for analysis of fats and oils by GC.

Here, using the pretreatment procedures introduced in the previous section for various types of processed foods, we investigated the use of FTIR as a screening technique for analysis of trans fatty acids.

3-1. Infrared Spectra of Unsaturated Fatty Acids

Fig. 3-1-1 shows the infrared spectra of triolein with a cis double bond, and trielaidin with a trans double bond. Fig. 3-1-2 shows the infrared spectra of the decomposition fatty acid products, methyl oleate and methyl elaidate, obtained through methylation of these triglycerides. A peak in the vicinity of 966 cm^{-1} is apparent in the infrared spectra of trielaidin and methyl elaidate. This is due to absorption associated with the trans-vinylene group =C-H out-of-plane bending, a peak that is characteristic of trans unsaturated fatty acids. In the case of the cis isomer, it is displayed as a broad peak below 800 cm^{-1}.

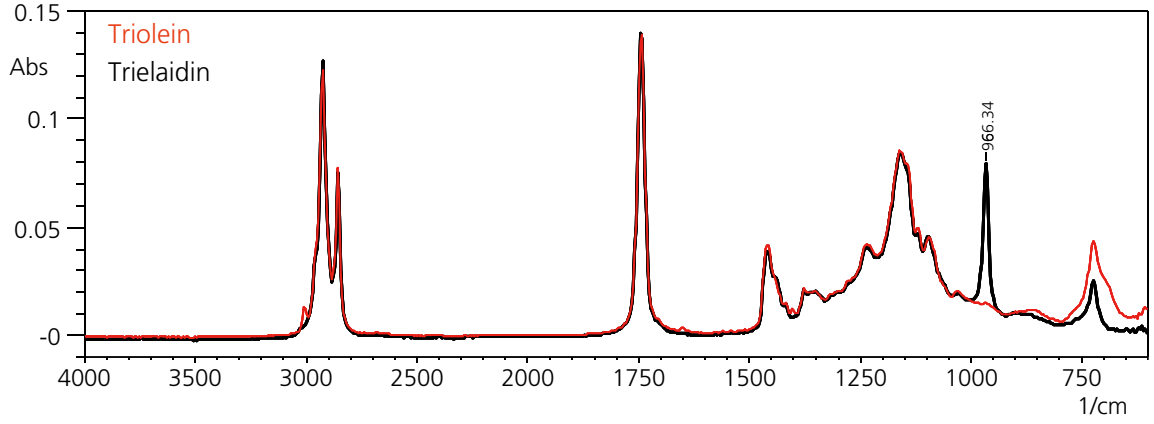

Fig. 3-1-1 Infrared Spectra of Triolein and Trielaidin Standards

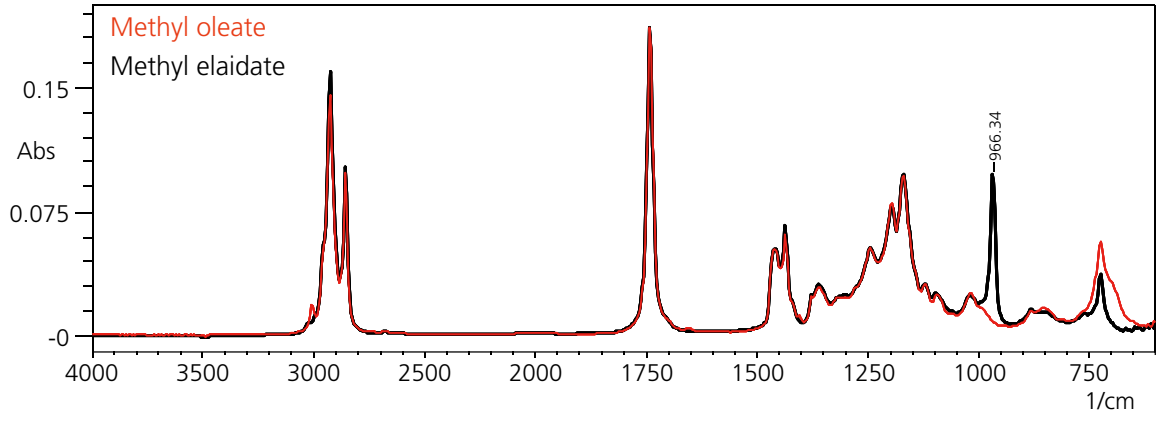

Fig. 3-1-2 Infrared Spectra of Methyl Oleate and Methyl Elaidate Standards

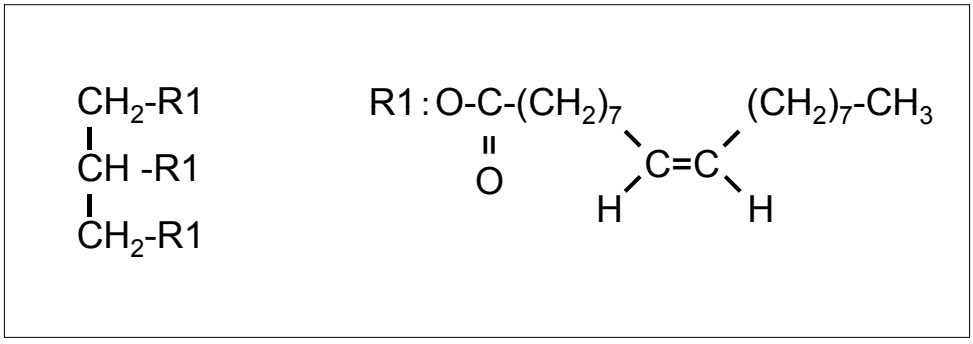

Fig. 3-1-3 Structural Formula of Triolein

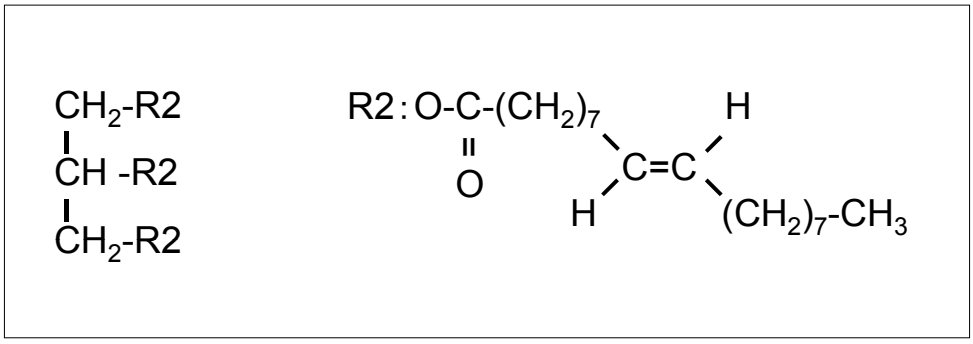

Fig. 3-1-4 Structural Formula of Trielaidin

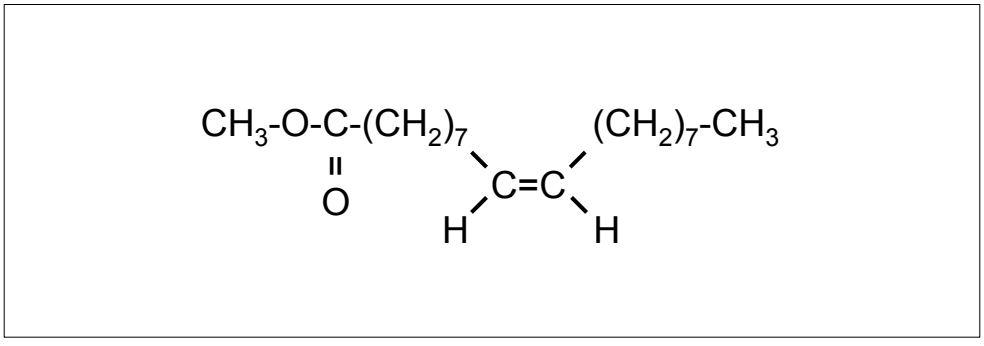

Fig. 3-1-5 Structural Formula of Methyl Oleate

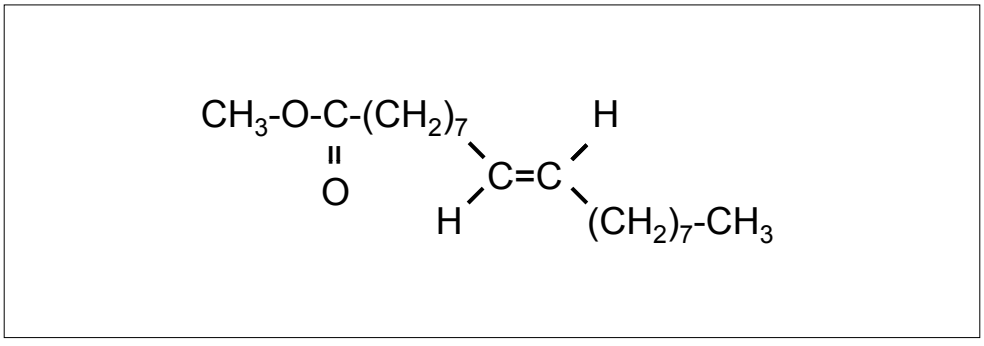

Fig. 3-1-6 Structural Formula of Methyl Elaidate

3-2. Calibration Curve for Methylated Fatty Acid by Infrared Absorption

To conduct quantitative analysis of the trans fatty acids in methylated samples, we prepared standard samples using methyl oleate and methyl elaidate, and then generated a calibration curve. The standards were prepared by adding methyl elaidate to methyl oleate to prepare solutions for generating a 7-point calibration curve, with methyl elaidate concentrations of 0.0 (unspiked), 0.1, 0.3, 0.5, 1.0, 5.0 and 10.0 % (v/v), respectively. Three repeat transmission measurements were conducted for each standard solution, using a KBr fixed cell with a 0.1 mm optical path length. The measurement results for each standard solution (one spectrum per standard) are shown in Fig. 3-2-1, and the calibration curve based on the area of the peak at 966 cm^{-1} is shown in Fig. 3-2-2. The measurement conditions are shown in Table 3-2-1. In addition, 10 repeat measurements of the 0.0 % concentration methyl elaidate standard were conducted, and the quantitation limit (10 σ) was determined from the standard deviation and slope of the calibration curve. The correlation coefficient R^2 = 0.999942 was obtained, indicating excellent calibration curve linearity, and the quantitation limit was 0.064 %.

3-3. Quantitation of Trans Fatty Acids in Processed Food by FTIR

After conducting the pretreatment operations described in the previous sections, including chloroform/methanol solvent extraction or saponification followed by methylation processing, the solvent was removed from the sample solutions, and quantitation of the trans fatty acids in the obtained methylated fatty acids was conducted. Some of the infrared spectra obtained from the measurements are shown in Fig. 3-3-1. The calibration curve of Fig. 3-2-2 was used for the quantitative calculations, and 5 repeat measurements were conducted for each sample using the conditions shown in Table 3-2-1. The quantitation results for the samples are shown in Table 3-3-1. These values indicate the concentrations of trans fatty acids in the fats and oils. Good repeatability was obtained even for samples having less than 1 % concentration of fats and oils.

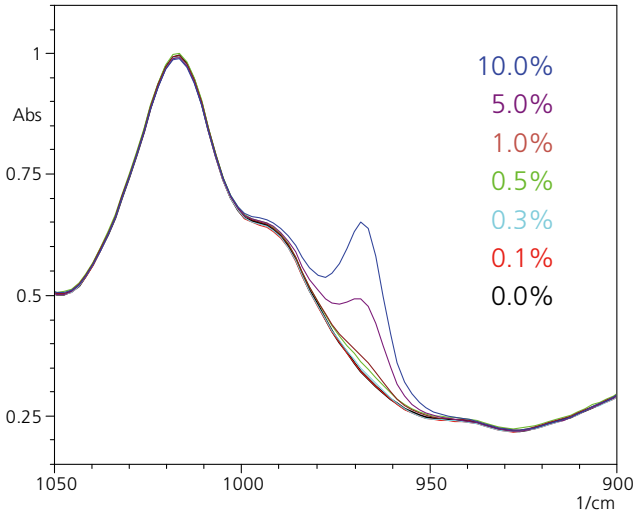

Fig. 3-2-1 Infrared Spectra of Standards

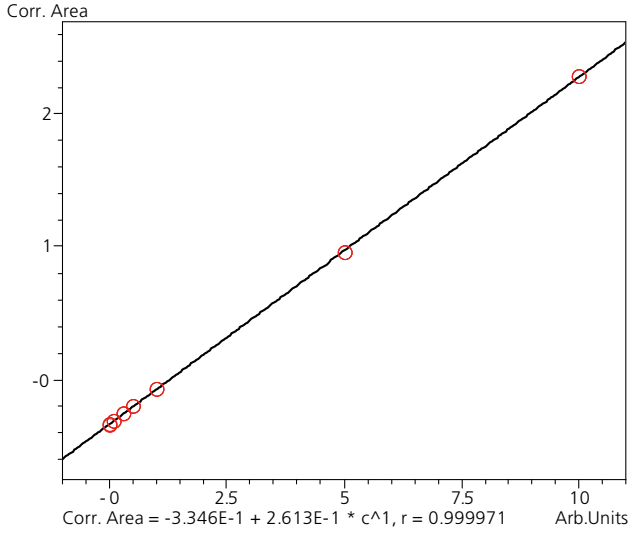

Fig. 3-2-2 FTIR Calibration Curve

Table 3-2-1 Measurement Conditions

Fourier Transform Infrared Spectrophotometer	
Analytical Instrument	: IRPrestige-21, fixed thickness cell (0.1 mm, KBr)
Resolution	: 4.0 cm^{-1}
Accumulation	: 20 scans (Approx. 30 seconds)
Detector	: DLATGS

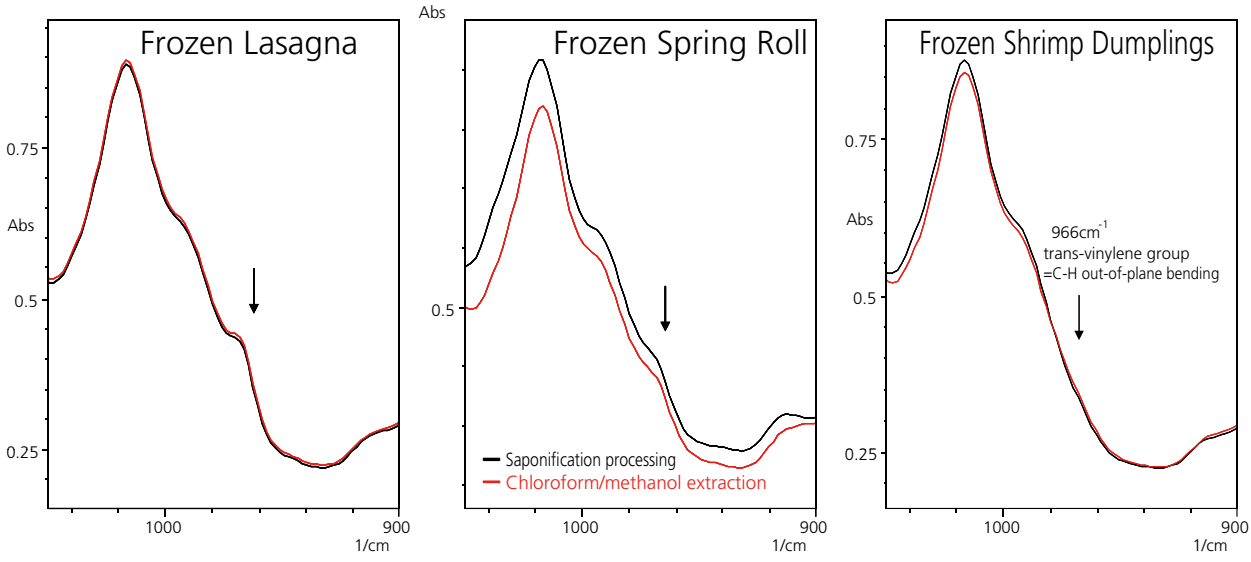

Fig. 3-3-1 Infrared Spectra of Processed Food Samples

Table 3-3-1 FTIR Quantitation Results for Trans Fatty Acids in Processed Food Samples (Concentrations in Fats and Oils)

	Frozen Pizza A	
	CHCl3/MeOH	Saponification
1	3.744	3.467
2	3.740	3.468
3	3.738	3.469
4	3.737	3.462
5	3.734	3.464
Average	3.739	3.466

	Frozen Pizza B	
	CHCl3/MeOH	Saponification
1	3.679	3.470
2	3.670	3.477
3	3.673	3.472
4	3.674	3.472
5	3.668	3.468
Average	3.672	3.472

	Frozen Pizza C	
	CHCl3/MeOH	Saponification
1	3.193	3.053
2	3.173	3.059
3	3.169	3.062
4	3.177	3.062
5	3.171	3.061
Average	3.177	3.059

	Frozen Lasagna	
	CHCl3/MeOH	Saponification
1	3.203	3.206
2	3.207	3.195
3	3.214	3.197
4	3.211	3.198
5	3.213	3.201
Average	3.210	3.199

	Frozen Shrimp Doria	
	CHCl3/MeOH	Saponification
1	3.205	2.947
2	3.200	2.939
3	3.203	2.938
4	3.207	2.936
5	3.208	2.936
Average	3.205	2.939

	Frozen Shrimp Gratin	
	CHCl3/MeOH	Saponification
1	2.939	2.850
2	2.936	2.858
3	2.933	2.860
4	2.940	2.859
5	2.940	2.858
Average	2.938	2.857

	Frozen Shrimp Dumplings	
	CHCl3/MeOH	Saponification
1	0.602	0.347
2	0.614	0.351
3	0.615	0.348
4	0.618	0.353
5	0.623	0.351
Average	0.614	0.350

	Frozen Spring Roll	
	CHCl3/MeOH	Saponification
1	2.132	2.030
2	2.151	2.036
3	2.152	2.036
4	2.153	2.035
5	2.153	2.037
Average	2.148	2.035

	Frozen Deep Fry	
	CHCl3/MeOH	Saponification
1	1.406	1.123
2	1.414	1.125
3	1.425	1.129
4	1.414	1.123
5	1.413	1.130
Average	1.414	1.126

	Beef Curry (Retort)	
	CHCl3/MeOH	Saponification
1	2.745	2.275
2	2.737	2.276
3	2.737	2.275
4	2.736	2.276
5	2.738	2.276
Average	2.738	2.276

	Potato Chips	
	CHCl3/MeOH	Saponification
1	0.626	0.470
2	0.645	0.441
3	0.641	0.442
4	0.646	0.442
5	0.639	0.450
Average	0.640	0.449

	Chocolate Chip Cookies	
	CHCl3/MeOH	Saponification
1	0.125	0.158
2	0.136	0.158
3	0.137	0.160
4	0.145	0.164
5	0.140	0.167
Average	0.136	0.161

(%)

3-4. Examination Using Second Derivative Processing

As is clear from the infrared spectrum of the methyl elaidate standard sample in methyl oleate shown in Fig. 3-1-2, and the measurement results of the pretreated processed foods shown in Fig. 3-3-1, the peaks associated with trans fatty acids in the vicinity of 966 cm^{-1} display overlapping absorption bands in the region from 1050–950 cm^{-1}. Second derivative processing is often used for confirmation and quantitation of this type of shoulder peak. Therefore, we applied second derivative processing to the measurement results obtained from the standard sample and the pretreated processed foods, and attempted to generate a calibration curve and perform quantitative calculations.

The second derivative processing results applied to the measurement results from the standard samples (1 spectrum per sample) are shown in Fig. 3-4-1. Peaks in second derivative processing point down. From the second derivative processing the absorption peaks in the vicinity of 966 cm^{-1} are readily apparent. Fig. 3-4-2 shows the calibration curve for the peak areas in the vicinity of 966 cm^{-1} which was generated following the application of second derivative processing, and the correlation coefficient R^2 became 0.999678. In addition, 10 repeat measurements of the 0.0 % concentration methyl elaidate standard were conducted, and the quantitation limit (10 σ) was determined from the standard deviation and slope of the calibration curve to be 0.065 %. These values are approximately the same as the correlation coefficient R^2 = 0.999942 and quantitation limit 0.064 % obtained directly, without using second derivative processing.

Table 3-4-1 shows the quantitation results for the processed foods obtained using second derivative processing. Compared to the values obtained directly, without second derivative processing (Table 3-3-1), better repeatability was obtained without conducting second derivative processing. It is presumed that this decrease in repeatability may be due to the tendency of second derivative processing to amplify noise.

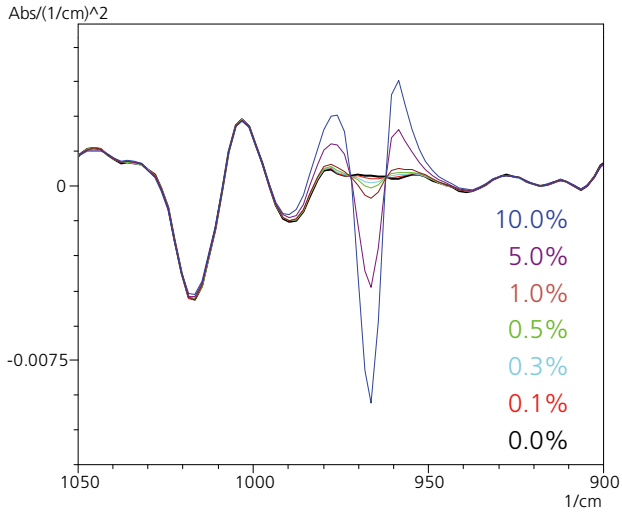

Fig. 3-4-1 Infrared Spectra of Standards After Second Derivative Processing

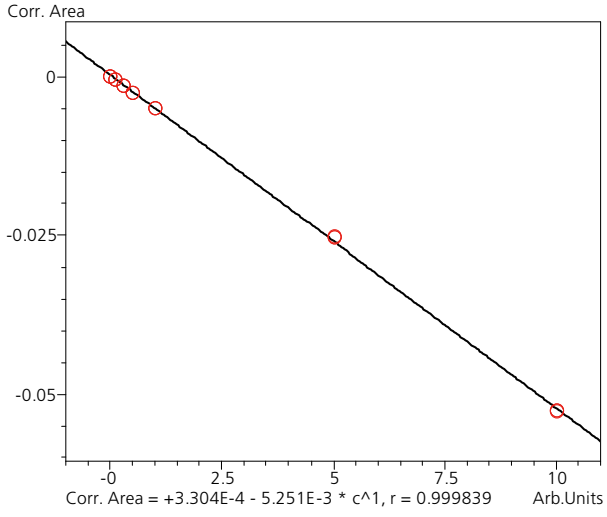

Fig. 3-4-2 FTIR Calibration Curve After Second Derivative Processing

Table 3-4-1 FTIR Quantitation Results for Trans Fatty Acids in Processed Food Samples After Second Derivative Processing (Concentrations in Fats and Oils)

	Frozen Pizza A	
	CHCl3/MeOH	Saponification
1	3.558	3.564
2	3.627	3.555
3	3.608	3.564
4	3.589	3.551
5	3.608	3.546
Average	3.598	3.556

	Frozen Pizza B	
	CHCl3/MeOH	Saponification
1	3.813	3.609
2	3.829	3.661
3	3.827	3.649
4	3.809	3.654
5	3.808	3.634
Average	3.817	3.641

	Frozen Pizza C	
	CHCl3/MeOH	Saponification
1	2.673	2.987
2	2.649	2.993
3	2.640	3.009
4	2.658	2.994
5	2.618	2.984
Average	2.647	2.993

	Frozen Lasagna	
	CHCl3/MeOH	Saponification
1	3.069	3.133
2	3.119	3.126
3	3.112	3.134
4	3.124	3.137
5	3.109	3.108
Average	3.107	3.127

	Frozen Shrimp Doria	
	CHCl3/MeOH	Saponification
1	2.947	3.135
2	2.931	3.125
3	2.952	3.156
4	2.953	3.145
5	2.932	3.150
Average	2.943	3.142

	Frozen Shrimp Gratin	
	CHCl3/MeOH	Saponification
1	3.058	3.032
2	3.066	3.012
3	3.062	3.026
4	3.067	3.011
5	3.069	3.015
Average	3.064	3.019

	Frozen Shrimp Dumplings	
	CHCl3/MeOH	Saponification
1	0.277	0.387
2	0.221	0.387
3	0.227	0.399
4	0.225	0.405
5	0.224	0.390
Average	0.235	0.394

	Frozen Spring Roll	
	CHCl3/MeOH	Saponification
1	1.416	1.519
2	1.303	1.522
3	1.303	1.537
4	1.304	1.530
5	1.280	1.519
Average	1.321	1.525

	Frozen Deep Fry	
	CHCl3/MeOH	Saponification
1	0.481	0.779
2	0.461	0.782
3	0.393	0.778
4	0.448	0.802
5	0.443	0.775
Average	0.445	0.783

	Beef Curry (Retort)	
	CHCl3/MeOH	Saponification
1	1.821	2.403
2	1.802	2.409
3	1.813	2.396
4	1.814	2.388
5	1.789	2.392
Average	1.808	2.397

	Potato Chips	
	CHCl3/MeOH	Saponification
1	0.321	0.442
2	0.310	0.439
3	0.314	0.443
4	0.312	0.443
5	0.277	0.443
Average	0.307	0.442

	Chocolate Chip Cookies	
	CHCl3/MeOH	Saponification
1	0.429	0.388
2	0.426	0.400
3	0.431	0.387
4	0.443	0.401
5	0.426	0.401
Average	0.431	0.396

(%)

3-5. Effect of Chain Length

In general, fatty acids that comprise fats and oils have a carbon chain structure in which the median number of carbons is 18, as they also include fatty acids having acyl chains with differing numbers of carbon atoms. The methyl and methylene absorption intensities differ depending on the type of fats and oils. The elaidic acid used as the trans fatty acid standard here has a carbon chain number of 18, and a single trans double bond. Trans fatty acids having different carbon chain numbers can possibly be considered to display different peak intensities. Here, we compared the intensities of the peaks in the vicinity of 966 cm^{-1} of elaidic acid and 2 other types of fatty acids having acyl chains with differing numbers of carbon atoms.

Fig. 3-5-1 shows the infrared spectra of methyl myristelaidate (C14), methyl palmitelaidate (C16), and methyl elaidate, obtained from measurement by single reflection ATR method. The measurement conditions are shown in Table 3-5-1. It is obvious that the infrared spectra of these 3 unsaturated fatty acid esters are nearly identical, and this can be attributed to their having the same structure, except for their carbon atom numbers. The differences in their peak methyl and methylene absorption intensities however are due to their different carbon chain lengths and are visibly evident. Fig. 3-5-2 shows a magnified view of the region of 966 cm^{-1} of Fig. 3-5-1, and Table 3-5-2 shows their respective peak area values (977.952–956.734 cm^{-1} average of 3 repeat measurements).

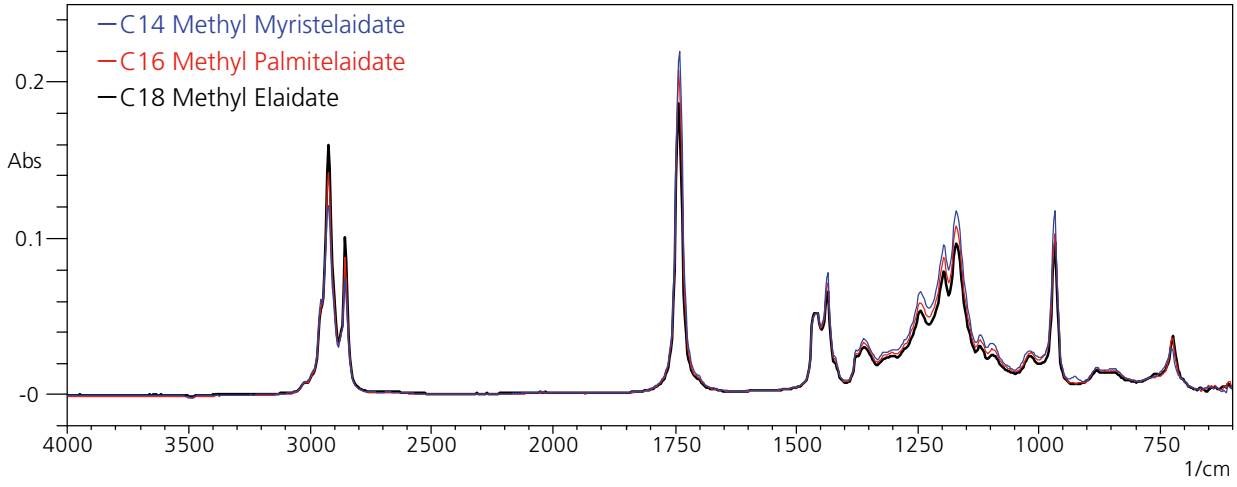

Fig. 3-5-1 Comparison of Infrared Spectra Based on C14, C16, C18 Carbon Chain Length (Single Reflection ATR Method)

Table 3-5-1 Measurement Conditions

Fourier Transform Infrared Spectrophotometer	
Analytical Instrument	: IRPrestige-21, MIRacle A (ZnSe)
Resolution	: 4.0 cm^{-1}
Accumulation	: 100 scans (Approx. 150 seconds)
Detector	: DLATGS

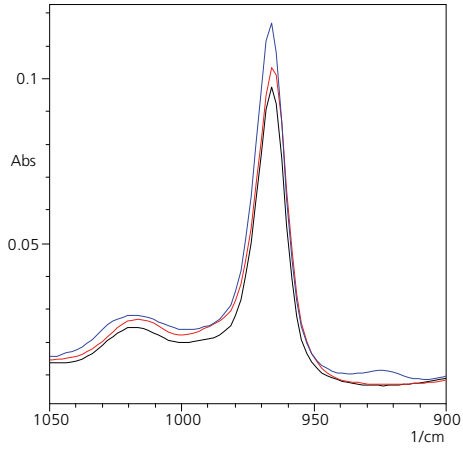

Fig. 3-5-2 Comparison of Infrared Spectra Based on Carbon Chain Length (Magnification)

Table 3-5-2 Carbon Chain Length Sensitivity Comparison

Spectrum	Chain Length	Peak Area	Peak Area Ratio
Methyl elaidate-1	C18	0.737	
Methyl elaidate-2	C18	0.741	
Methyl elaidate-3	C18	0.743	
Average		0.740	
Methyl palmitelaidate-1	C16	0.750	
Methyl palmitelaidate-2	C16	0.750	
Methyl palmitelaidate-3	C16	0.750	C16/C18
Average		0.750	1.0133
Methyl myristelaidate-1	C14	0.887	
Methyl myristelaidate-2	C14	0.886	
Methyl myristelaidate-3	C14	0.884	C14/C18
Average		0.885	1.1958

From these results, it is understood that compared to the peak area of methyl elaidate, the peak areas of methyl palmitelaidate, with carbon chain number 16, and methyl myristelaidate, with carbon chain number 14, are approximately 1 % and 20 % greater, respectively.
Therefore, as the carbon chain number of the unsaturated fatty acid chain of fats and oils becomes smaller, the error in the trans fatty acid quantitation value based on elaidic acid appears to increase. Conversely, as the carbon chain number of an unsaturated fatty acid becomes greater, it is presumed that there is a decrease in the error included in the trans fatty acid quantitation value.

3-6. Quantitation of Trans Fatty Acids in Lipids (Triglycerides)

Unlike processed foods, which contain a wide range of substances, fat and oil products contain almost nothing but lipids, and can therefore be measured directly by FTIR without any pretreatment such as extraction or methylation. Even if the lipids have a triglyceride structure, the characteristic peak of trans unsaturated fatty acids appears in the vicinity of 966 cm^{-1}. As shown in Fig. 3-1-1, this peak intensity can be used to conduct quantitation of the trans fatty acid.

We prepared standard samples using triolein and trielaidin, and then generated a calibration curve. The standards were prepared by adding trielaidin to triolein to prepare solutions for generating a 7-point calibration curve, with trielaidin concentrations of 0.0 (unspiked), 0.1, 0.3, 0.5, 1.0, 5.0 and 10.0 % (w/w), respectively. Three repeat transmission measurements were conducted for each standard solution, using a KBr fixed cell with a 0.1 mm optical path length. The measurement results for each standard solution (one spectrum per standard) are shown in Fig. 3-6-1, and the calibration curve based on the area of the peak at 966 cm^{-1} is shown in Fig. 3-6-2. The measurement conditions were the same as those of Table 3-2-1. In addition, 10 repeat measurements of the 0.0 % concentration trielaidin standard were conducted, and the quantitation limit (10 σ) was determined from the standard deviation and slope of the calibration curve. The correlation coefficient R^2 = 0.999918 was obtained, indicating excellent calibration curve linearity, and the quantitation limit was 0.060 %.

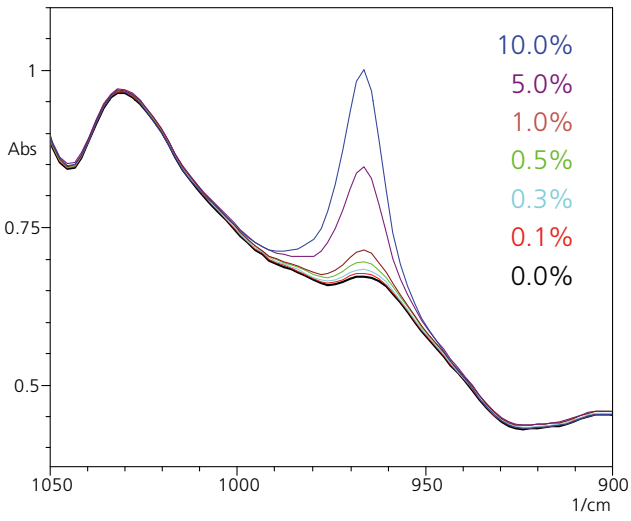

Fig. 3-6-1 Infrared Spectra of Triglyceride Standards

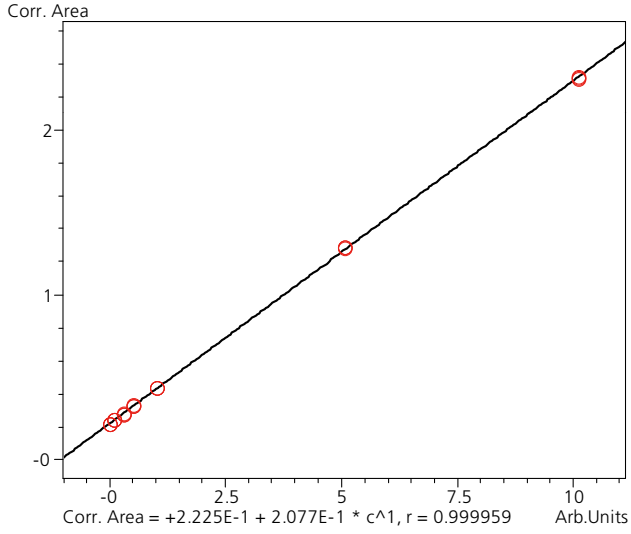

Fig. 3-6-2 FTIR Calibration Curve of Trielaidin

4. Analysis by GC/FID

Analysis of trans fatty acids in processed foods using GC is conducted by first extracting the fats and oils, then preparing the methylated fatty acids, and finally separating the components using a polar cyanopropyl capillary column. However, even using a highly polar column, many of the isomers in the sample may not be completely separated, and quantitation may be difficult due to the various contaminants present in processed foods. Furthermore, chlorinated solvents such as chloroform and methanol that are used in common solvent extraction methods have been put into question due to their heavy environmental impact. Therefore, eliminating the use of chlorinated solvents, here we directly conducted saponification of a processed food sample, and examined this simple pretreatment method for recovering the methylated fatty acids by comparing the results with those obtained using the extraction method.

4-1. Calibration Curve by GC/FID

To conduct quantitation of methylated trans fatty acids that were obtained as a result of pretreatment of processed food samples, standards were prepared using methyl elaidate, and a calibration curve was generated. For the standards, methyl elaidate was diluted with n-hexane to generate a 4-point calibration curve, with methyl elaidate concentrations of 0.05, 0.1, 0.5, and 1 mg/mL, respectively. Methyl heptadecanoate (17:0) was added to each of the sample solutions as an internal standard at a concentration of 1 mg/mL. Fig. 4-1-1 shows the chromatograms of the standard samples, and Fig. 4-1-2 shows the generated calibration curve. The analytical conditions are shown in Table 4.1.1. Excellent linearity was obtained over the entire concentration range of the calibration curve, with a coefficient of correlation R^2 = 0.999987. Due to the difficulty in preparing standards for all of the trans fatty acid isomers, quantitation was conducted by applying the coefficient obtained from the elaidic acid calibration curve to the other trans fatty acid peaks. Because the sensitivity of the FID varies depending on the carbon number, for the substances having different carbon numbers, carbon number correction was conducted using 18 + 1 = 19 as a proportional calculation based on the methyl elaidate (C 18:1) carbon number, and the total trans fatty acid quantity was calculated. For example, the quantitative value of a C 16:1 methylated trans fatty acid is corrected by multiplying that value by 19/ (16 + 1).

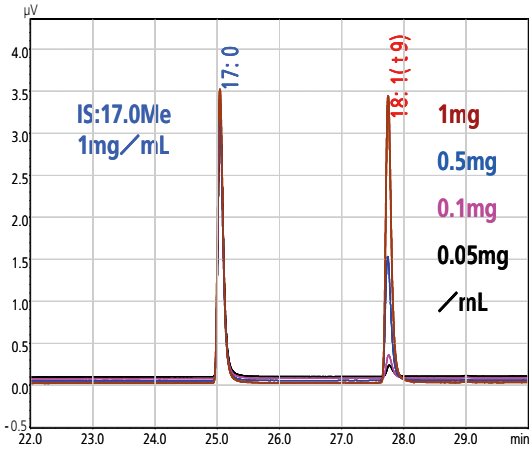

Fig. 4.1.1 Chromatograms of Standard Samples

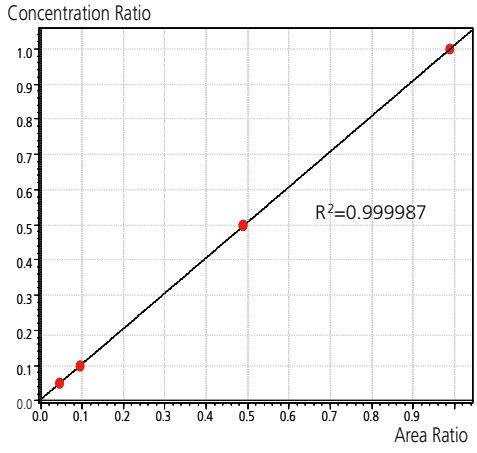

Fig. 4.1.2 Elaidic Acid Calibration Curve

Table 4.1.1 GC/FID Analytical Conditions

Gas Chromatograph

Instrument	: GC-2010 Plus	Injection Mode	: Split
Column	: BPX-90 (0.25 mm I.D. × 100 m d.f., 0.25 μm)	Split Ratio	: 30:1
Column Temp.	: 120 °C (2 min) – 3 °C/min – 250 °C (10 min)	FID Temp.	: 260 °C
INJ Temp.	: 250 °C	Sample Size	: 1 μL
Carrier Gas	: He (20 cm/sec)		

4-2. Comparison of GC/FID Chromatograms of Actual Samples Using Different Pretreatment Methods

The GC/FID chromatograms obtained from analysis of several processed foods were compared based on whether pretreatment was conducted by typical solvent extraction using chloroform/methanol or by saponification. The results indicated that there was not a big difference in the GC chromatogram profiles due to the pretreatment method used. Thus, we confirmed that saponification, which does not rely on the use of high-environmental-impact chlorinated solvents, can be used without any problem in analysis of processed foods.

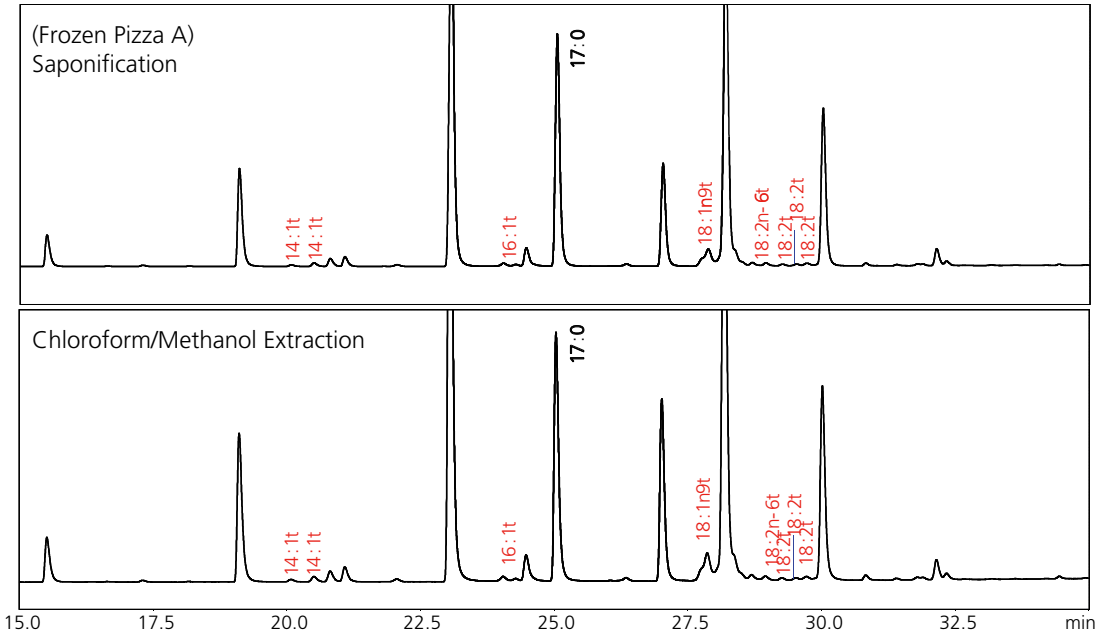

Fig. 4-2-1 Comparison of Results due to Pretreatment Method for Frozen Pizza A

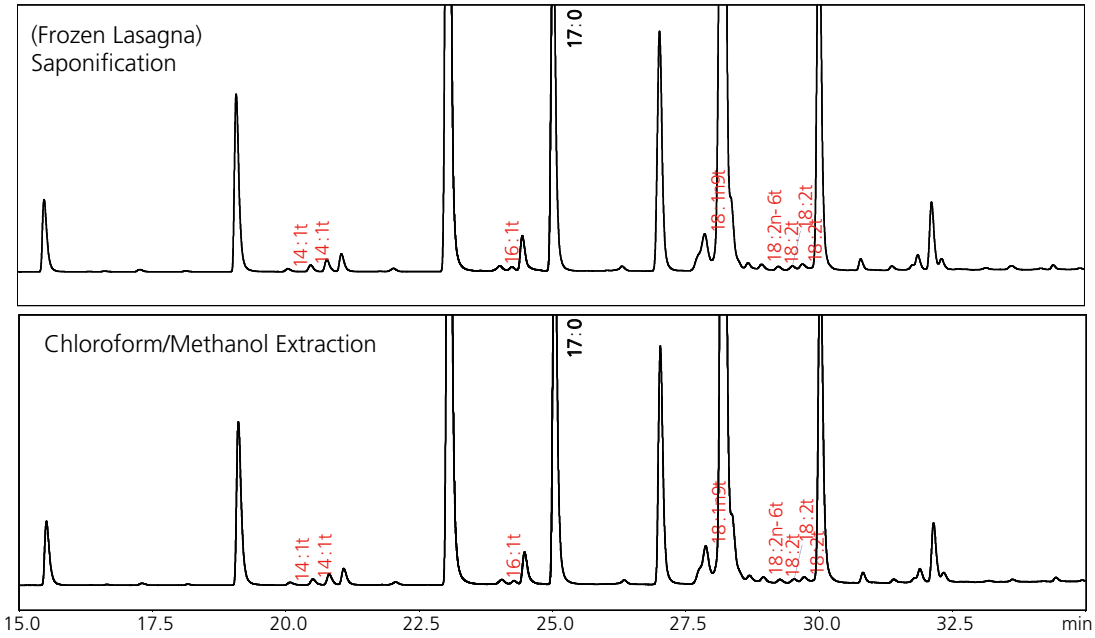

Fig. 4-2-2 Comparison of Results due to Pretreatment Method for Frozen Lasagna

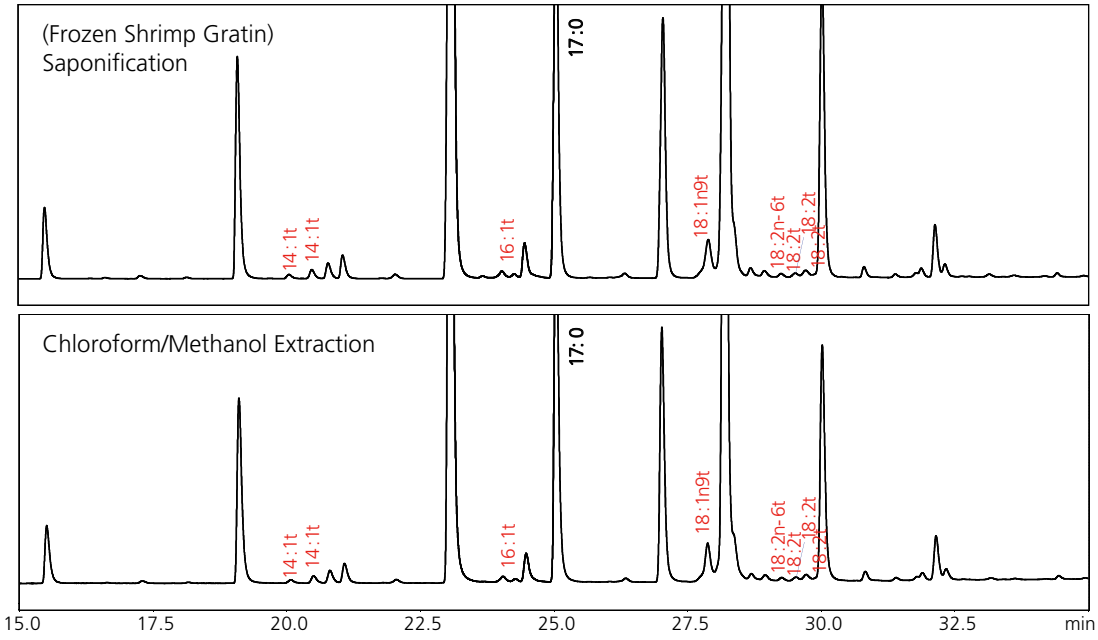

Fig. 4-2-3 Comparison of Results due to Pretreatment Method for Frozen Shrimp Gratin

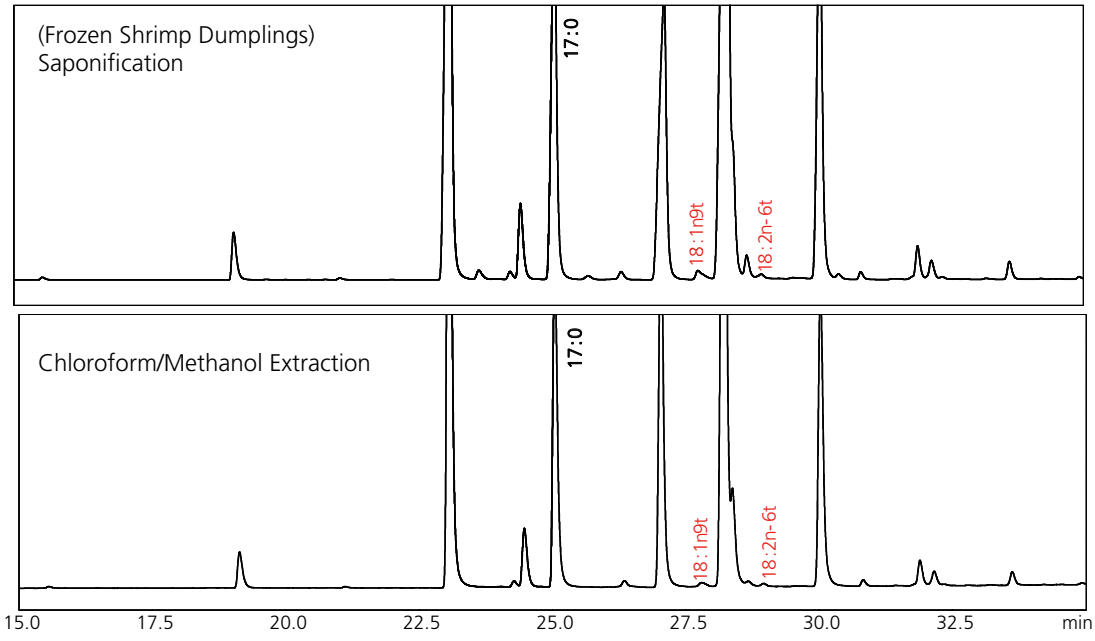

Fig. 4-2-4 Comparison of Results due to Pretreatment Method for Frozen Shrimp Dumplings

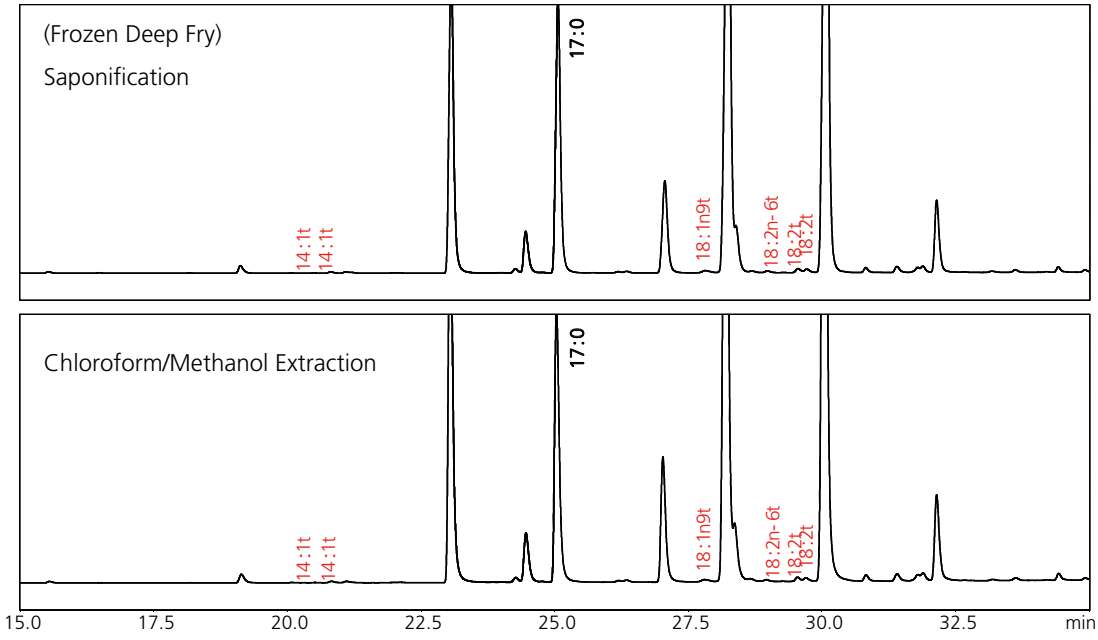

Fig. 4-2-5 Comparison of Results due to Pretreatment Method for Frozen Deep Fry

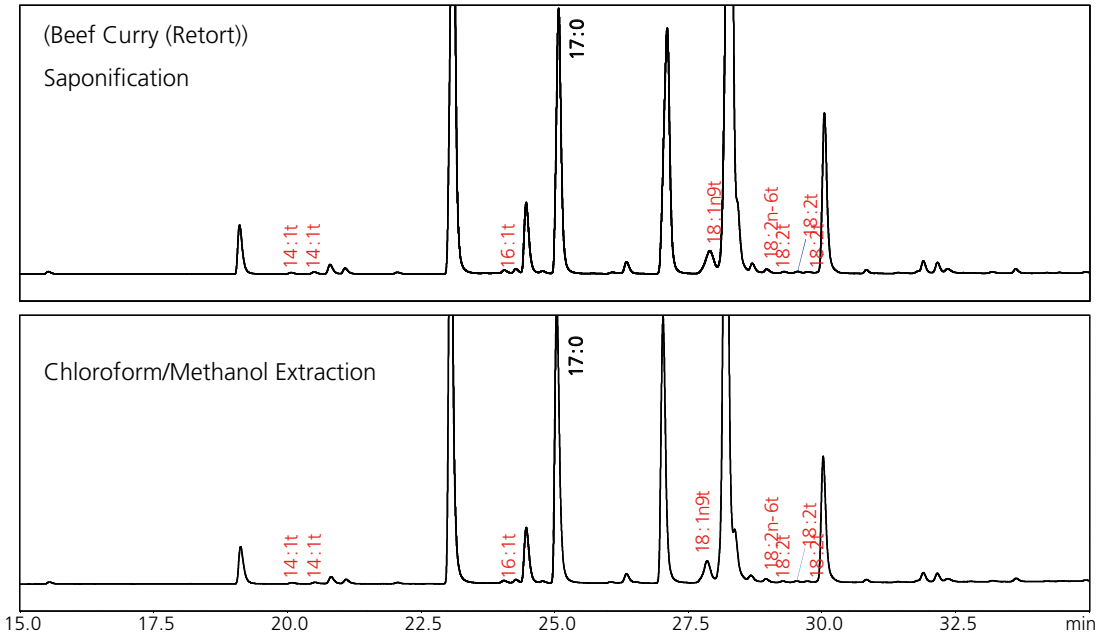

Fig. 4-2-6 Comparison of Results due to Pretreatment Method for Beef Curry (Retort)

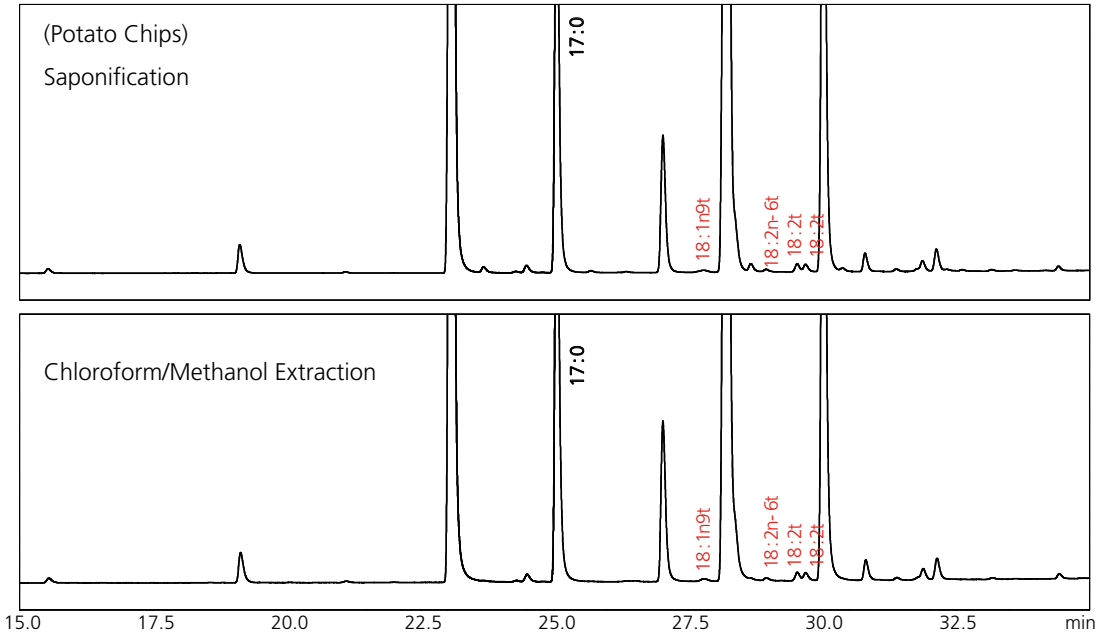

Fig. 4-2-7 Comparison of Results due to Pretreatment Method for Potato Chips

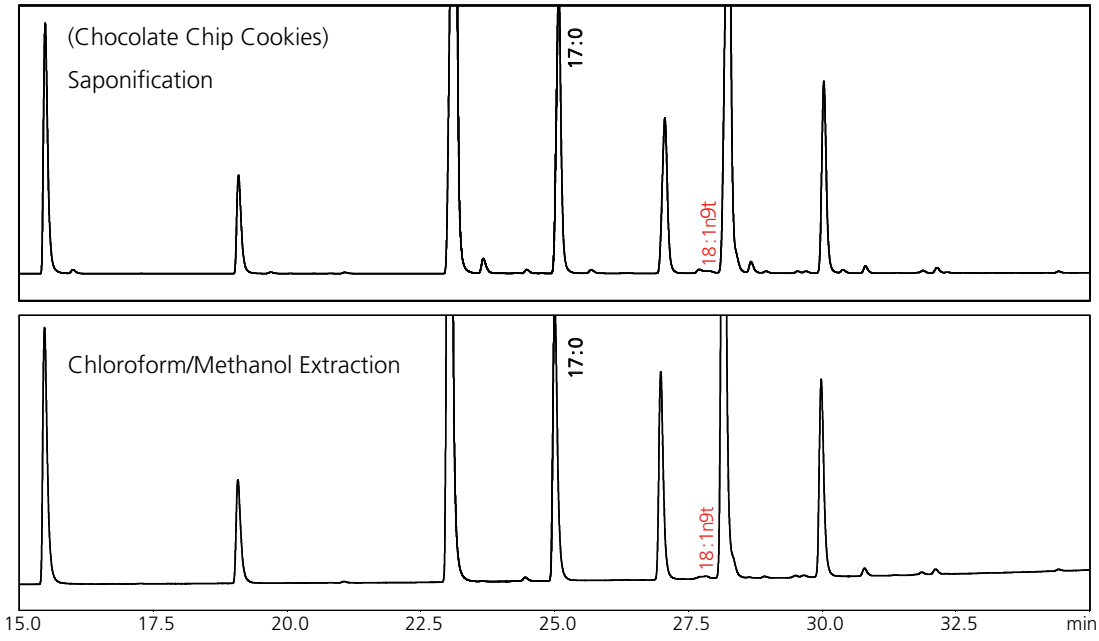

Fig. 4-2-8 Comparison of Results due to Pretreatment Method for Chocolate Chip Cookies

4-3. Fractionation by Silver Nitrate Column

To select peaks to use for quantitation of the trans fatty acids, a silver nitrate column cartridge, which can separate the cis and trans isomers of the methylated sample, was used to separate the isomers into three distinct fractions, as shown in the operation flow of Fig. 4-3-1.
Comparing the chromatograms shown in Fig. 4-3-2, and specifically comparing the trans-monoenoic fatty acid methyl esters and saturated fatty acid methyl esters in fraction 1 and the cis-monoenoic fatty acids in fraction 2, we selected trans-monoenoic fatty acid peaks for quantitation. For the dienoic fatty acids, we selected the dienoic fatty acid methyl ester peak eluted before the 18:2n-6c methyl esters (cis, cis) in the fraction 3 chromatogram as the trans acid.

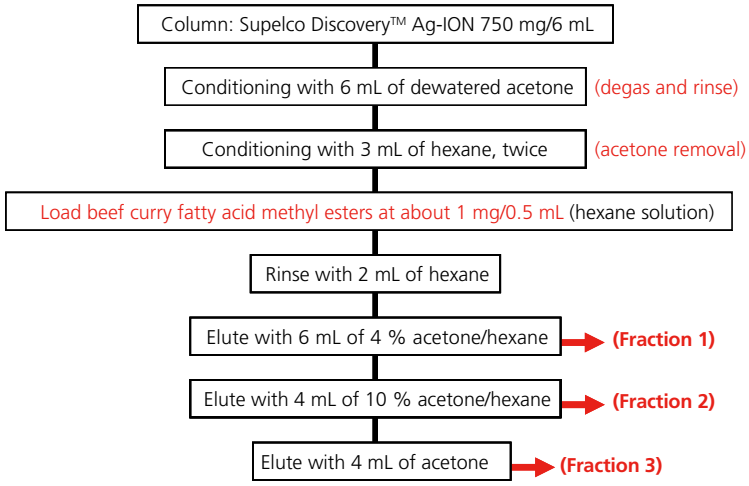

Fig. 4-3-1 Fractionation Flow Using Silver Nitrate Column

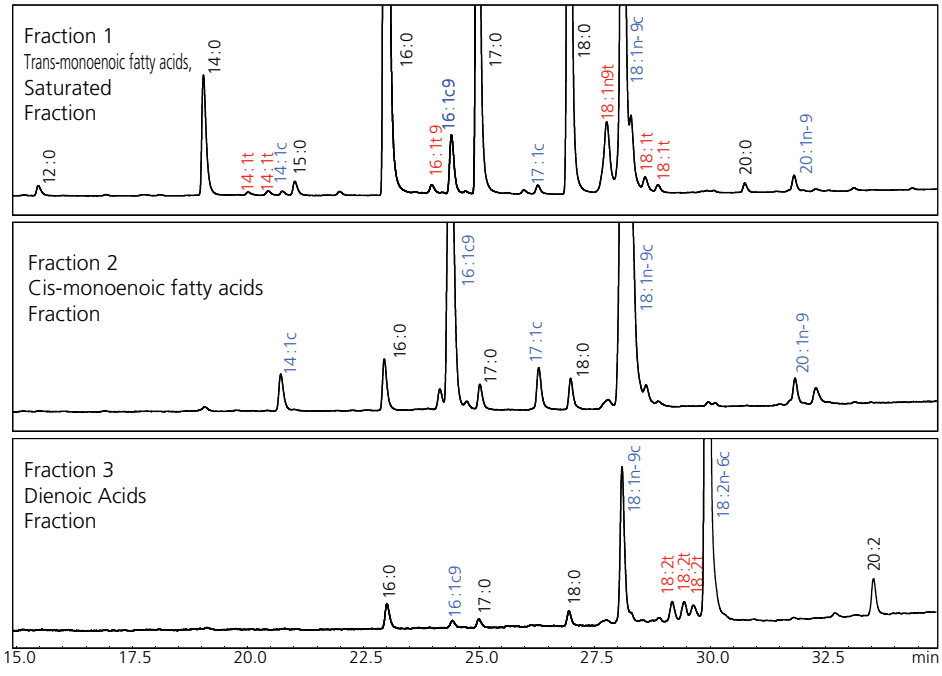

Fig. 4-3-2 Fractionation of Beef Curry Using Silver Nitrate Column

4-4. Quantitation of Trans Fatty Acids in Processed Foods by GC

After conducting pretreatment of the processed foods using chloroform/methanol solvent extraction and saponification, respectively, we conducted quantitation of the trans fatty acids in each of the sample solutions. For the calculation, the coefficient obtained from the methyl elaidate calibration curve of Fig. 4.1.2 was applied to all of the methylated trans fatty acid peaks, and quantitation was performed by conducting the sensitivity correction for all substances with carbon numbers different from that of elaidic acid, as described in Section 4-1. The quantitation results for all of the samples are shown in Table 4-4-1. The quantitation values reflect the trans fatty acid concentrations in each of the fats and oils.

There was not a big difference in quantitation values with respect to the use of chloroform/methanol solvent extraction or saponification. Thus, we were able to confirm that with the processed foods examined here, saponification, which relies primarily on the use of hexane, can be used just as effectively as the problematic chlorinated organic solvents like chloroform, that are typically required for solvent extraction.

Table 4-4-1 GC Quantitation Results for Trans Fatty Acids in Processed Food Samples (Concentrations in Fats and Oils)

	Frozen Pizza A	
	CHCl3/MeOH	Saponification
1	3.666	2.767
2	3.615	2.782
Average	3.641	2.774

	Frozen Pizza B	
	CHCl3/MeOH	Saponification
1	3.550	3.438
2	3.514	3.435
Average	3.532	3.436

	Frozen Pizza C	
	CHCl3/MeOH	Saponification
1	3.065	3.065
2	2.995	3.183
Average	3.030	3.124

	Frozen Pizza A	
	CHCl3/MeOH	Saponification
1	3.049	3.677
2	3.092	3.637
Average	3.071	3.657

	Frozen Pizza B	
	CHCl3/MeOH	Saponification
1	3.090	3.172
2	3.146	3.162
Average	3.118	3.167

	Frozen Pizza C	
	CHCl3/MeOH	Saponification
1	2.842	2.634
2	2.815	2.616
Average	2.828	2.625

	Frozen Pizza A	
	CHCl3/MeOH	Saponification
1	0.377	0.517
2	0.353	0.458
Average	0.365	0.487

	Frozen Pizza B	
	CHCl3/MeOH	Saponification
1	1.454	1.431
2	1.369	1.457
Average	1.412	1.444

	Frozen Pizza C	
	CHCl3/MeOH	Saponification
1	0.857	0.839
2	0.836	0.837
Average	0.846	0.838

	Frozen Pizza A	
	CHCl3/MeOH	Saponification
1	2.333	2.467
2	2.335	2.465
Average	2.334	2.466

	Frozen Pizza B	
	CHCl3/MeOH	Saponification
1	0.670	0.690
2	0.663	0.696
Average	0.666	0.693

	Frozen Pizza C	
	CHCl3/MeOH	Saponification
1	0.298	0.324
2	0.296	0.329
Average	0.297	0.327

(%)

5. Comparison of Quantitation Values Obtained by FTIR and GC

The quantitation values for the trans fatty acids determined by FTIR (Table 3-3-1) and GC/FID (Table 4-4-1) are displayed and compared in Table 5-1. No significant difference was observed between the quantitative values determined by GC/FID and FTIR.

Table 5-2 shows the weights of the total fat measured following chloroform/methanol extraction from each sample, and the total fat content as listed on each of the product packages. The package-listed quantities and actual measured values showed few differences, and were considered to be within the range of variation due to non-uniformity of the food.

The total fat measurement results shown in Table 5-2, and the trans fatty acid weight per 100 g of food determined from the quantitative values in fat (Table 5-1) by FTIR and GC are shown in Table 5-3. Fig. 5-1 shows the quantitative value correlation graphs generated according to the different sample pretreatment methods, and Fig. 5-2 shows the quantitative value correlation graphs associated with FTIR and GC analyses.

Table 5-1 Comparison of GC/FID and FTIR Quantitation Results for Trans Fatty Acids in Processed Food Samples (Concentrations in Fats and Oils)

	Frozen Pizza A	
	CHCl3/MeOH	Saponification
GC	3.641	2.774
FTIR	3.739	3.466

	Frozen Pizza B	
	CHCl3/MeOH	Saponification
GC	3.532	3.436
FTIR	3.672	3.472

	Frozen Pizza C	
	CHCl3/MeOH	Saponification
GC	3.030	3.124
FTIR	3.177	3.059

	Frozen Lasagna	
	CHCl3/MeOH	Saponification
GC	3.071	3.657
FTIR	3.210	3.199

	Frozen Shrimp Doria	
	CHCl3/MeOH	Saponification
GC	3.118	3.167
FTIR	3.205	2.939

	Frozen Shrimp Gratin	
	CHCl3/MeOH	Saponification
GC	2.828	2.625
FTIR	2.938	2.857

	Frozen Shrimp Dumplings	
	CHCl3/MeOH	Saponification
GC	0.365	0.487
FTIR	0.614	0.350

	Frozen Spring Roll	
	CHCl3/MeOH	Saponification
GC	1.412	1.444
FTIR	2.148	2.035

	Frozen Deep Fry	
	CHCl3/MeOH	Saponification
GC	0.846	0.838
FTIR	1.414	1.126

	Beef Curry (Retort)	
	CHCl3/MeOH	Saponification
GC	2.334	2.466
FTIR	2.738	2.276

	Potato Chips	
	CHCl3/MeOH	Saponification
GC	0.666	0.693
FTIR	0.640	0.449

	Chocolate Chip Cookies	
	CHCl3/MeOH	Saponification
GC	0.297	0.327
FTIR	0.136	0.161

(%)

Table 5-2 Total Fat Content in Processed Food Samples

	Frozen Pizza A	Frozen Pizza B	Frozen Pizza C
Measured Result	7.3	9.6	8.1
Package Listed Values	6.8	11.0	10.6

	Frozen Lasagna	Frozen Shrimp Doria	Frozen Shrimp Gratin
Measured Result	5.5	4.5	5.8
Package Listed Values	6.9	5.7	6.6

	Frozen Shrimp Dumplings	Frozen Spring Roll	Frozen Deep Fry
Measured Result	8.9	20.0	10.2
Package Listed Values	8.7	16.8	10.5

	Beef Curry (Retort)	Potato Chips	Chocolate Chip Cookies
Measured Result	5.5	37.5	28.3
Package Listed Values	5.5	35.0	27.9

(g)

Table 5-3 Comparison of GC/FID and FTIR Quantitation Results for Trans Fatty Acids in Processed Food Samples (Weight per 100 g Food)

	Frozen Pizza A	
	CHCl3/MeOH	Saponification
GC	0.266	0.203
FTIR	0.273	0.253

	Frozen Pizza B	
	CHCl3/MeOH	Saponification
GC	0.339	0.330
FTIR	0.353	0.333

	Frozen Pizza C	
	CHCl3/MeOH	Saponification
GC	0.245	0.253
FTIR	0.257	0.248

	Frozen Lasagna	
	CHCl3/MeOH	Saponification
GC	0.169	0.201
FTIR	0.177	0.176

	Frozen Shrimp Doria	
	CHCl3/MeOH	Saponification
GC	0.140	0.143
FTIR	0.144	0.132

	Frozen Shrimp Gratin	
	CHCl3/MeOH	Saponification
GC	0.164	0.152
FTIR	0.170	0.166

	Frozen Shrimp Dumplings	
	CHCl3/MeOH	Saponification
GC	0.032	0.043
FTIR	0.055	0.031

	Frozen Spring Roll	
	CHCl3/MeOH	Saponification
GC	0.282	0.289
FTIR	0.430	0.407

	Frozen Deep Fry	
	CHCl3/MeOH	Saponification
GC	0.086	0.085
FTIR	0.144	0.115

	Beef Curry (Retort)	
	CHCl3/MeOH	Saponification
GC	0.128	0.136
FTIR	0.151	0.125

	Potato Chips	
	CHCl3/MeOH	Saponification
GC	0.250	0.260
FTIR	0.240	0.168

	Chocolate Chip Cookies	
	CHCl3/MeOH	Saponification
GC	0.084	0.092
FTIR	0.039	0.046

(g)

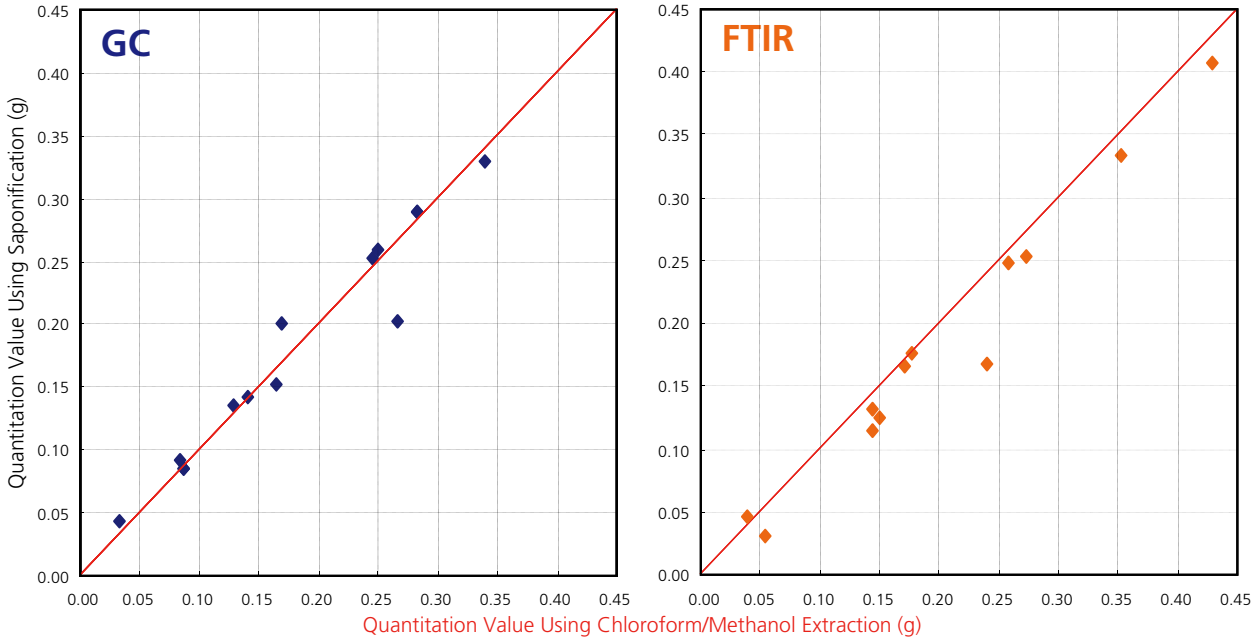

Fig. 5-1 Correlation of Trans Fatty Acid Quantitation Values According to Sample Pretreatment Method (Weight per 100 g Food)

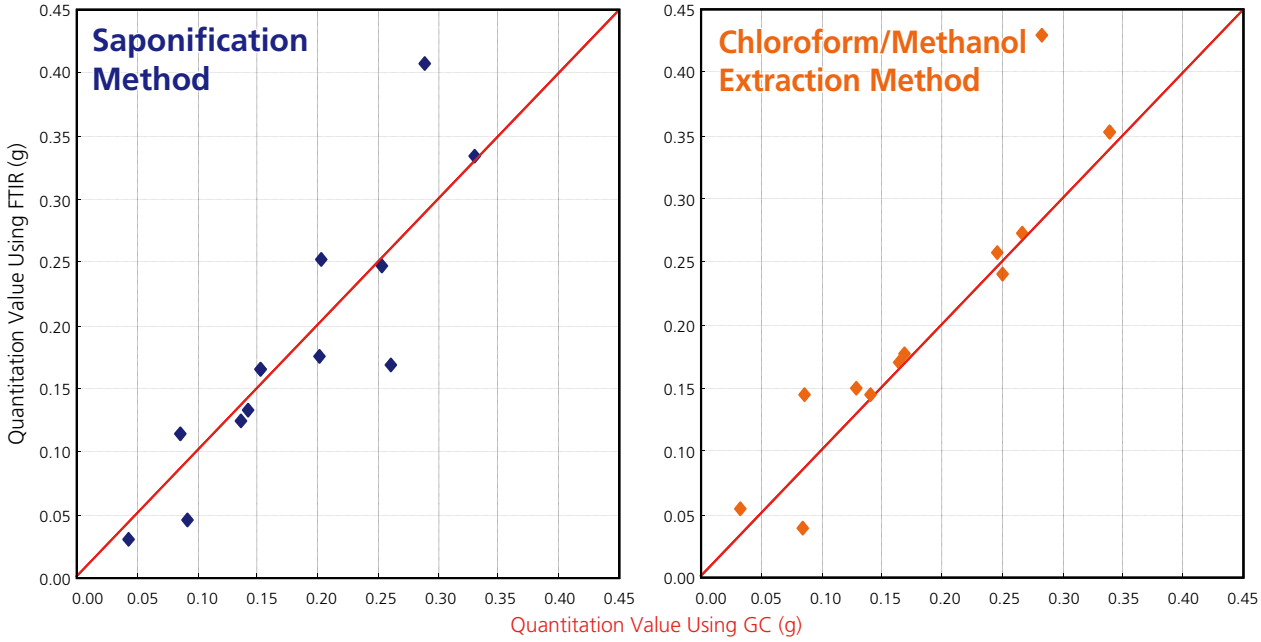

Fig. 5-2 Correlation of Trans Fatty Acid Quantitation Values Using FTIR and GC/FID (Weight per 100 g Food)

6. Summary

In this investigation regarding the quantitation of trans fatty acids in processed foods, there was not a great difference in the patterns of the FTIR spectra and GC/FID chromatograms when comparing chloroform/methanol extraction and saponification as pretreatment methods. Approximately the same quantitation values were obtained using simple saponification, an extremely useful technique, instead of the conventional solvent extraction method, which relies on solvents like chloroform that have a large environmental impact. In actual sample analysis, also, the trans fatty acid quantitation values obtained using GC/FID and FTIR showed good correlation, and it was confirmed that quantitation of methylated trans fatty acids by FTIR was achieved without any interference due to contaminants.

Using FTIR, the multiple trans double bonds in dienoic acid and trienoic acid, etc. increase the absorption intensities, which has a positive influence on the quantitative values because the sensitivity of short carbon chain fatty acids is higher than that of the standard used for the calibration curve. Also, since the sensitivity of long carbon chain fatty acids is lower than that of the calibration curve standard, there is a negative influence on quantitation values.

With the GC/FID method, in addition to the possible adverse effect on the quantitation of trans fatty acids due to the small trans isomer peaks and the un-separated cis isomer peaks, large errors can be introduced due to mis-identification. Therefore, it is necessary to check the cis and trans isomer separation profile by conducting fractionation using a silver nitrate column, etc. for different types of food samples.

Considering that both FTIR and GC/FID can be used for quantitation of methylated fatty acids, and that their generated quantitation values show good correlation, it is reasonable to suggest that because of the big difference in measurement time, FTIR may be a more effective technique for screening. However, in cases where FTIR analysis yields a quantitative result that is near the standard value, re-measurement by GC/FID would likely be more efficient.

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