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Analysis of *Trans* Fat in Edible Oils with Cooking Process

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Trans fat is a unsaturated fatty acid with *trans* configuration and separated double bonds. Analytical methods have been introduced to analyze *trans* fat content in foods including infrared (IR) spectroscopy, gas chromatography (GC), Fourier transform-infrared (FT-IR) spectroscopy, reverses-phase silver ion high performance liquid chromatography, and silver nitrate thin layer chromatography. Currently, FT-IR spectroscopy and GC are mostly used methods. *Trans* fat content in 6 vegetable oils were analyzed and processing effects including baking, stir-frying, pan-frying, and frying on the formation of *trans* fat in corn oil was evaluated by GC. Among tested vegetable oils, corn oil has 0.25 g *trans* fat/100 g, whereas other oils including rapeseed, soybean, olive, perilla, and sesame oils did not have detectable amount of *trans* fat content. Among cooking methods, stir-frying increased *trans* fat in corn oil whereas baking, pan-frying, and frying procedures did not make changes in *trans* fat content compared to untreated corn oils. However, the *trans* fat content was so low and food label can be declared as '0' *trans* based on the regulation of Ministry of Food ad Drug Safety (MFDS) (< 2 g/100 g edible oil).

Key words: *Trans* fat, Vegetable oil, Processing procedure, Fatty acid, Gas chromatography

INTRODUCTION

Trans fat is unsaturated fatty acid with *trans* configuration rather than *cis* configuration, which is a normal isomer in naturally occurring lipids. However, polyunsaturated fatty acids having double bonds with conjugated forms such as conjugated linoleic acid are not categorized as *trans* fat due to their health beneficial influence. Hydrogenation, which uses hydrogen gas and metal catalysts to reduce the degree of unsaturation and provide proper physical characteristics in oils since 1890s, is a major process to generate *trans* fat in our daily diet. Margarine, shortening, and butters are well-known solid fat containing *trans* isomers. Other sources of *trans* fat in our daily diet are microorganisms in the rumens of ruminant animals through biohydrogenation. *Roseburia hominis A2-183T*, *Roseburia inulinivorans A2-192T* and *Ruminococcus obeum-like strain A2-162* can produce *trans* fatty acids from ruminant sources (1,2). Dairy products including meat, milk, and butter possess about 2~5% of *trans* fat due to the activities of microorganisms in ruminant animals such as cows and goats (3).

The profiles of *trans* fat from commercially available partially hydrogenated oils and natural resources are not the same. Vaccenic acid (C18:1n11*t*), which has *trans* structure in carbon 11 of octadecenoic acid, is major monounsaturated *trans* fat from biohydrogenation whereas partially hydrogenated oils by chemical hydrogenation have more elaidic acid (C18:1n9*t*), which has *trans* part in carbon 9 of octadecenoic acid (1,2).

Detrimental health implication of *trans* fat diet has been filed up for more than four decades. Epidemiologic and biochemical evidence confirmed that excessive *trans* fats in the diet are a significant risk factor for cardiovascular disease. Health effects of *trans* fat could be due to the structures of *trans* fat (1,2). The melting point of stearic acid (C18:0), oleic acid (C18:1n9*c*), and elaidic acid (C18:1n9*t*), are 70, –5, and 42°C, respectively, which implies that *trans* fat exists as solid at room temperature and human body temperature like saturated fat.

A number of methods have been introduced to determine total *trans* fat content including infrared (IR) spectroscopy,

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gas chromatography (GC), Fourier transform-infrared (FT-IR) spectroscopy, reverses-phase silver ion high performance liquid chromatography (HPLC), and silver nitrate thin layer chromatography ($AgNO_3$-TLC) (4).

IR region around 967 cm^{-1} can detect an isolated *trans* double bond due to the deformation of C-H bonds adjacent to the *trans* double bond. Official methods of AOCS, AOAC, and IUPAC adapted the measurement of the absorption intensity for total *trans* content in triacylglycerols, methyl esters of unesterified fatty acids. However, this IR method has some limitations. Triacylglycerols can absorb the region of 970 cm^{-1} and mono- and di-*trans* conjugated double bonds absorb at about 950 and 980, and 990 cm^{-1}, respectively, which could interfere with isolated *trans* fat content. This method can be useful in oils with less than 5% conjugated fatty acids due to the interference of absorption by conjugated bonds and in oils with 5% or higher content of *trans* fat due to the low intensity of IR absorption (5).

The advanced techniques for *trans* fat analysis in fats and oils are using FT-IR spectroscopy. FT-IR has more advantages including high signal to noise ratio (S/N), high wavelength calibration accuracy (reference He-Ne laser), improved light throughput and speed of analysis (Michelson interferometer), rapid and comprehensive data collection than IR spectroscopy on determining total *trans* fat. FT-IR technique has better accuracy in oils with less than 5% *trans* fat through adjustment of baselines for example samples with more than 10% *trans* fat at 944 and 988 cm^{-1}, less than 10% *trans* fat at 944 and 985 cm^{-1}, and below 5% *trans* fat at 944 and 973 cm^{-1} (6). These FT-IR methods used carbon disulfide like IR method, which may lead to stratification, vapor and air bubbles within cells. A Michelson Interferometer in FT-IR can help all the wavelength of IR pass through samples simultaneously. FT-IR can determine the content of *trans* fat with high correlation coefficient without using carbon disulfide. *trans* Peak areas can be integrated from 945 to 990 cm^{-1} and quantification can be done through fitting the measured *trans* areas with a second order polynomial (7). Official method of AOCS and AOAC using the FT-IR can be used for quantitative determination of *trans* fat above 1 and 5%, respectively. Some disadvantages of FT-IR method for determining *trans* fat are overestimating the content of *trans* fat, interference from conjugated fatty acids, and the expressing results as equivalent of elaidic acid (C18:1 n9*t*) content (4).

The next advance in *trans* fat analysis by FT-IR spectroscopy came from using attenuated total reflection-Fourier transform-infrared spectroscopy (ATR-FT-IR). When light strikes two different medium at normal condition, some light partially transmitted and partially reflected. However, in internal reflection mode, light is reflected inside the crystal and evanescent wave is then propagating away from the surface of the crystal like *trans* fat. The intensity of this wave is partly attenuated by IR and this internal reflection is known as ATR.

ATR-FT-IR has been introduced to measure *trans* fat in oils and fat samples using middle IR range and the ATR cell. This technique requires prior extraction of fat from food samples while further derivatization and use of harmful solvents were not necessary (8). Priego-Capote *et al.* (9) used ATR-IR spectroscopy for determination of total fat and *trans* fatty-acids content in bakery products. Da Costa Filho (3) develop a method determining *trans* fatty acids in edible oils using ATR-FT-IR over a wavelength range of 600-4000 cm^{-1} and the peak height at 966.3 cm^{-1} was electronically converted to its negative second derivative for *trans* fat content. This ATR-FT-IR can determine less than 1% trans fat in edible oils and fats including palm, peanut, soybean, and sunflower oils.

GC for the analysis of fatty acid methyl esters (FAME) are the most convenient and accurate method. Therefore, GC methods can isolate *trans* configuration from *cis* forms, which can not be achieved by IR or FT-IR spectroscopy methods (10). Conventional GC for analysis of FAME use a slightly polar column using polyglycol Carbowax-20 because natural oils have majorly *cis* form of double bonds. Separation of *trans*/*cis* configuration requires more polar stationary phase including SP-2560, SP-2340, OV-275, BPX-70 or CP-SIL-88 using highly polar cyanosilicone. Recently, the length of capillary columns extended to 100 or 120 m to make better resolution if the isolation of *cis*/*trans* isomers (4). American Oil Chemists Society (AOCS) (11) approved official method Ce 1h-05 for the determination of *cis*, *trans*-saturated, monounsaturated, and polyunsaturated fatty acids in vegetable or non-ruminant animal oils and fats by capillary GC with a flame ionization detector (FID). Also, Official Method of Analysis 996.06 revised by the AOAC International (AOAC) adapted 100-m high-polarity capillary column as a stationary phase for GC-FID method (10).

Different detectors like mass spectrometry (MS) coupled with GC were introduced for *trans* fat analysis (12). However, overlaps and co-elution of *cis*/*trans* isomers observed in chromatograms from GC-FID.

In case of foods from ruminant animals like dairy products, different extraction or GC conditions are needed due to a large number of short chained fatty acids and the variety of different molecular structures (13).

A combination of GC and IR methods was suggested by Ratnayake *et al.* (14). Total *trans* fat measured by IR can be corrected using a formula: IR trans = %18:*t* + 0.84 x %18:2*tt* + 1.74 x %18:3*t*, which can be obtained from GC analysis for *trans* FAME. The combination of GC and IR methods are useful to determine trans fat content in partially hydrogenated vegetable oil containing more than 5% trans fat. Cruz-Hernandez *et al.* (15) analyzed conjugated linoleic acids and *trans*-18:1 isomers in dairy fats by using a combination of GC, silver-ion TLC/GC, and silver-ion liquid chromatography.

Reversed-phase HPLC can separate fatty acids based on the apparent carbon number, which is the number of carbons - 2x number of double bonds. This technique cannot separate *cis*/*trans* isomers in monounsaturated fatty acids whereas can separate linoleic acid isomers from non-octadecadienoic acids or conjugated linoleic acid. Silver salts can form more stable complex with *cis* isomers and $AgNO_3$ rather than *trans* isomer. This properties can be used to differentiate *cis*/*trans* isomers and applied to TLC, HPLC, and solid phase extraction columns (4).

Analysis of fatty acid profiles in one of typical procedures in food industry because providing information on the content of *trans* fat should be included in food label. Accurate information on the content of *trans* fat in foods can come from proper techniques for extracting lipids, saponification, and methylation of fatty acids. Each of above steps may include error sources for the manual operation, parameter settings, and/or data interpretations by the analysts (10).

Several official methods have been approved by any official national or international entity. As more accurate and precise methods and technologies are developed, some official methods are not frequently used whereas some are still valid. In Korea, officially approved analysis methods have been suggested in Korea Food Code. All the government agencies and laboratories from food industry should use this official method to certify the content of trans fat in the products.

Many reports have conducted on the fatty acid profiles and *trans* fat content in edible oils. However, effects of conventional cooking procedures including baking, stir-frying, pan-frying, and frying on the formation of *trans* fat in edible oils are scarce in the literature. Especially, vegetable oils from real cooking conditions for Total Dietary Study (TDS) in Korea have not been tried.

The objective of this study was to analyze *trans* fat content in commonly consumed vegetable oils and to find out effects of conventional cooking treatment on the formation of *trans* fat.

MATERIALS AND METHODS

Materials. Triundecanoin, elaidic acid, mixtures of standard fatty acids, and BF_3 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals were purchased from Daejung Chemical Co. (Seoul, Korea). Vegetable oils including corn, rapeseed, soybean, olive, perilla, and sesame oils were obtained from 18 large glossary markets located in 9 different cities in Korea for the purpose of Korean TDS from the year of 2013 to the year of 2016. The selected 9 cities were Seoul, Busan, Incheon, Daegu, Daejeon, Gwangju, Ulsan, Changwon, and Suwon, which had more than 1 million population. Best-selling branded vegetable oils, which were purchased from 18 different sampling sites, were sampled equal amount and blended to make a pool of oil. Each oil sample was provided in triplicate with separated bottles.

Sample preparation. Vegetable oils and corn oils with cooking procedures were kindly treated by Korea Health Industry Development Institute (KHIDI) based on the procedures of Korea Health and Nutrition Examination Survey conducted from the year of 2008 to the year of 2011. Cooking processes were selected as baking, stir-frying, pan-frying, and frying by KHIDI. Briefly, baking procedure was done using a preheated pan (diameter of 25 cm) at 180°C. Corn oil was spread in a preheated pan with a thin layer and maintained at 180°C for 1~2 min. Stir-frying procedure for corn oil was done in the preheated pan at 170°C. Corn oil in the pan was stirred continuously by a spatula for 2 min. For pan-frying procedure, corn oil was poured in the preheated pan at 170°C and was stirred 2 or 3 times per min by the spatula for 2 min. Frying was done using a shallow pan (diameter of 30 cm and height of 20 cm). Corn oil was filled in the shallow pan at the height of 10 cm and heated at 180°C. The corn oil in the shallow pan was further heated for 2 more min at 180°C. Corn oils sample with different cooking procedure were prepared in separated 3 bottles.

Fatty acid analysis by gas chromatography with a flame ionization detector (FID). Triundecanoin (C11:0), an internal standard, was dissolved in *n*-hexane and added to the extracted oil to the concentration of 1,000 ppm (w/v) and solvent was removed under nitrogen gas flow. Fatty acids were derivatized to fatty acid methyl esters (FAME) using BF_3/MeOH (14% boron trifluoride) and analyzed by GC according to AOAC 969.33 (16) with some modification. FAME was analyzed by Hewlett-Packard 6890 gas chromatograph (Agilent Technologies) with a FID, and a SP-2560 column (100 m × 0.25 mm ID, 0.20 μm film) from Supelco (Bellefonte, PA, USA). The oven temperature started at 100°C for 4 min, increased to 225°C at 3°C/min, and held at 225°C for 20 min. The temperatures of injector and detector were 225 and 285°C, respectively. The flow rate of helium carrier gas was 0.75 mL/min, the injection volume was 1 μL, and the split ratio was 1:200. Peaks of GC chromatograms were identified comparing the retention times of a mixture of standard fatty acid methyl esters (Sigma-Aldrich). Each peak of fatty acid was quantified using an equivalent of the concentration of the internal standard. Samples were separately analyzed in triplicate.

RESULTS AND DISCUSSION

Chromatograms of fatty acid standard mixture (a) and corn oil (b) are shown in Fig. 1. The retention times for internal standard (C11:0), elaidic acid (C18:1n9*t*), and *trans* linoleic acid (C18:2n6*t*) were 25.71, 43.68, and 45.12 min, respectively. Mixtures of standard fatty acids were well sep-

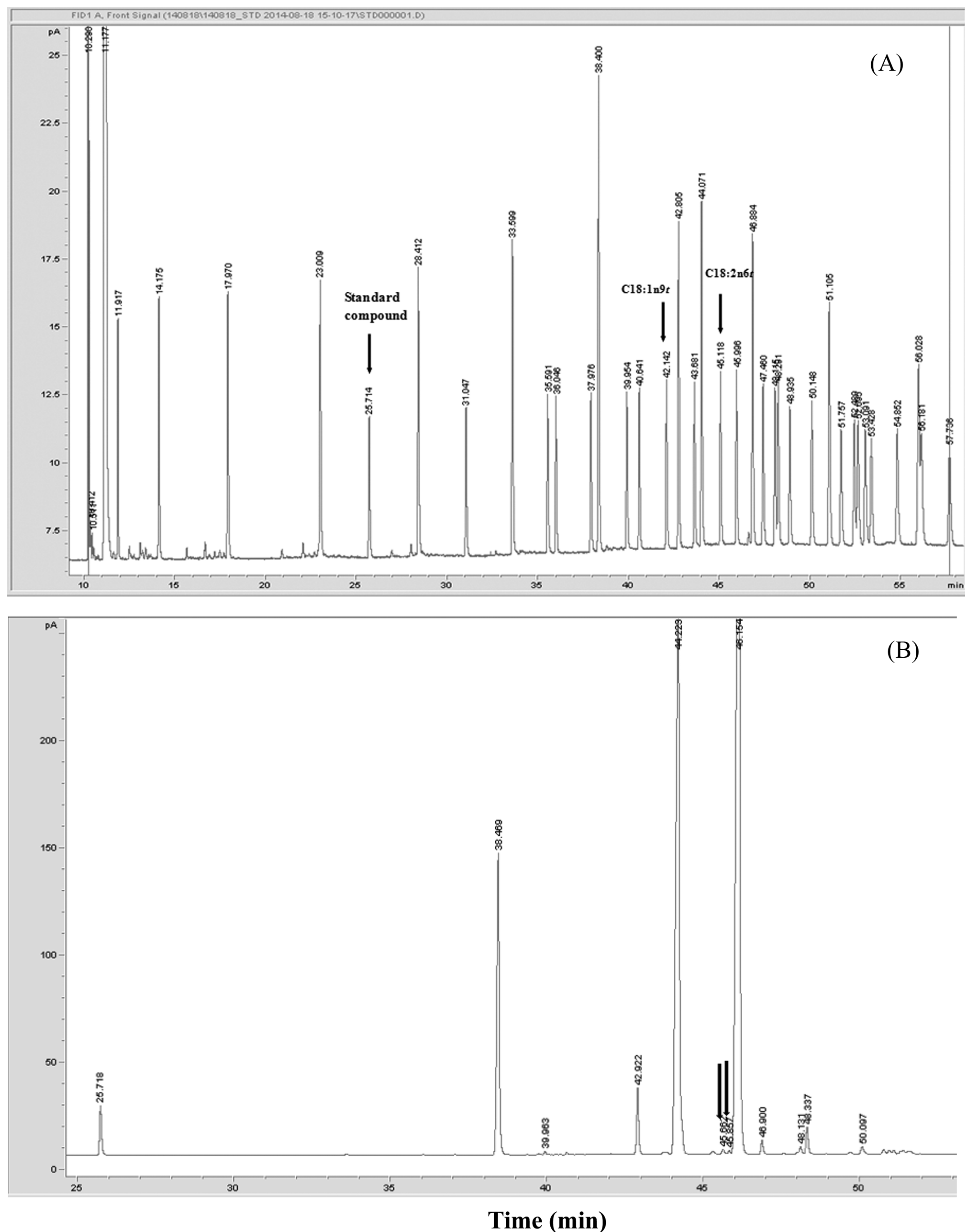

Fig. 1. Chromatograms of fatty acid standard mixture (A) and of corn oil (B). Arrows indicate the peaks of *trans* fatty acids.

arated using current analysis method.

Fatty acid profiles of 5 edible oils are shown in Table 1. Fatty acid profiles in vegetable oils were different substantially. Corn oil had high linoleic acid (50.28 g/100 g), followed by oleic acid (31.32 g/100 g), palmitic acid (12.65 g/100 g), and stearic acid (2.24 g/100 g). Rapeseed oils had oleic acid (68.20 g/100 g), followed by linoleic acid (15.38 g/100 g), linolenic acid (7.70 g/100 g) and palmitic acid (5.69 g/100 g). Soybean oil had high linoleic acid (45.23 g/100 g), followed by oleic acid (27.03 g/100 g), palmitic acid (16.05 g/100 g), and linolenic acid (6.09 g/100 g). Olive oil had the high oleic acid (78.11 g/100 g) as expected whereas perilla oil had high linolenic acid (57.78 g/100 g).

Corn oil had 0.25 g C18:2n6*t*/100 g whereas other oils including rapeseed, soybean, olive, perilla, and sesame oils did not possess *trans* fat. Presence of *trans* fat in corn oil

Table 1. Major fatty acid profiles and *trans* fat content in vegetable oils (g/100 g)

Sample	Fatty acid										*Trans* fatty acid	
	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0	C18:1n9*t*	C18:2n6*t*
Corn oil	12.65 ± 0.15[1]	0.11 ± 0.00	2.24 ± 0.02	31.32 ± 0.34	50.28 ± 0.61	0.93 ± 0.02	0.52 ± 0.00	0.23 ± 0.00	0.18 ± 0.14	N.D.[2]	N.D.	0.25 ± 0.01
Rapeseed oil	5.69 ± 0.13	N.D.	2.28 ± 0.01	68.20 ± 0.24	15.38 ± 0.12	7.70 ± 0.08	N.D.	0.75 ± 0.08	N.D.	N.D.	N.D.	N.D.
Soybean oil	16.05 ± 0.02	N.D.	5.59 ± 0.04	27.03 ± 0.01	45.23 ± 0.08	6.09 ± 0.02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Olive oil	13.65 ± 0.01	0.75 ± 0.00	3.38 ± 0.02	78.11 ± 0.05	3.61 ± 0.02	0.49 ± 0.02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Perilla seed oil	6.87 ± 0.08	0.22 ± 0.00	2.13 ± 0.02	19.71 ± 0.15	10.71 ± 0.14	57.78 ± 0.27	0.16 ± 0.00	0.12 ± 0.00	N.D.	N.D.	N.D.	N.D.
Sesame oil	10.58 ± 0.60	N.D.	6.18 ± 0.39	41.41 ± 2.94	33.83 ± 1.73	N.D.	0.64 ± 0.03	N.D.	N.D.	0.08 ± 0.14	N.D.	N.D.

[1]Mean ± standard deviation (n = 3).
[2]Not detected.

can be found in other previous reports. Zhang *et al.* (17) analyzed C18 *trans* fatty acids in edible oils including corn, sunflower, and olive oils using GC-mass spectrometry and found out that 1.29 g *trans* fat/100 g FAME in corn oil. C18:2 and C18:3 *trans* fat were almost equally responsible for the *trans* fat in corn oil (17). *Trans* fat in 19 corn oil in China was all detected ranging from 0.56 to 4.75 g/100 g with average of 2.01 g/100 g (18). Mossoba *et al.* (19) analyzed fatty acid profiles in edible oils including canola, coconut, corn, flax, grapeseed, olive, peanut, safflower, shortening, sunflower, and walnut oils using FT-NIR and GC methods and found out 0.36 to 0.99% *trans* fat in corn oil by GC method and 1.2 to 1.4% *trans* fat in corn oil by FT-NIR method. A value of 0 g *trans* fat per serving can be labeled if *trans* fat content is below 0.5 g per serving in US. Average serving size of oils in US is 14 g, which corresponds to a *trans* fat content of < 3.6% in the US. The detected 0.25% *trans* fat in corn oil is less than limitation and can be declared as 0 *trans* fat in food label.

Effects of cooking methods on the changes of *trans* fat content in corn oils are shown in Table 2. Processing including baking, stir-frying, pan-frying, and frying used thermal energy and lipid oxidation occurred in corn oils. Among cooking methods, stir-frying produced higher *trans* fat in corn oil than new corn oils whereas baking, pan-frying, and frying procedures did not make changes among *trans* fat content in corn oils. High temperature treatment can cause the formation of *trans* fat in oils through oxidation process. Żyżelewicz *et al.* (20) reported the formation of elaidic *trans* fat (C18:1n9*t*) in cocoa buffer extracted from roasted beans when roasting temperature was 135°C and roasting duration was 15 min. Tsuzuki *et al.* (21) confirmed the significant increases in *trans* fatty acids in edible oils during frying and heating process. When sliced raw potatoes were fried at 180°C, frying oils from potato frying had higher *trans* fatty acid than oils with the same temperature heating without potato frying (21).

Table 2. Effects of cooking process on *trans* fat content in corn oil (g/100 g)

Processing method	*Trans* fatty acid	
	C18:1n9*t*	C18:2n6*t*
Raw	N.D.[1]	0.25 ± 0.01[2]
Baking	N.D.	0.26 ± 0.01
Stir-frying	0.48 ± 0.32	0.56 ± 0.16
Pan-frying	N.D.	0.27 ± 0.01
Frying	N.D.	0.26 ± 0.01

[1]Not detected.
[2]Mean ± standard deviation (n = 3).

Trans formation from *cis* configuration in unsaturated lipids is inevitable steps during autoxidation, which is one of basic lipid oxidation mechanisms. Autoxidation is known as a free radical chain reaction with initiation, propagation, and final steps. Initial step is a formation of lipid radicals (L•) by losing hydrogen atom from unsaturated lipid (LH). Double bonds in *cis* configuration are unstable and tend to form *trans* configuration, which is more stable form than *cis* form (22). The higher temperature accelerates the reaction rates of oxidation and the more *trans* fat is generated.

Among tested cooking methods, stir-frying procedure at 170°C heating and continuous stirring process may accelerate the rates of lipid oxidation and formation of *trans* fat compared to other cooking procedures. However, the content of *trans* fat in vegetable oil after stir-frying process was far below the *trans* fat level compared to other food products like margarine made of partially hydrogenated oils (23). The content of *trans* fat in edible oils was so low and food label can be declared as '0' *trans* fat based on the regulation of Ministry of Food ad Drug Safety (MFDS). In

case of edible oils, *trans* fat content below 2 g per 100 g oil can be declared as '0' *trans*.

In conclusion, GC-FID is a reliable and accurate technique for the analysis of *trans* fat in vegetable oils. Among conventional cooking methods, stir-frying step can increase *trans* fat content in edible oils due to the high temperature treatment and continuous stirring, which might accelerate the rates of lipid oxidation. This GC-FID technique can be applied to any food matrix with proper lipid extraction methods for the quantification of *trans* fat content.

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