

Effect of Frying Temperature and duration on the Formation of Trans Fatty Acids in Selected Fats and Oils

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Abstract *Trans* fatty acids occur in food either naturally or produced during heat processing of food containing unsaturated fats. Naturally occurring *trans* fatty acids have different physiological and biological functions as compared to those formed in heat processed food which increase the risk of coronary heart disease. The aim of this study was to investigate the effect of heat treatments [heating temperature: 120, 150, 190 and 250 °C and heating period: 10, 30, 60 and 180 minutes] on the amount of *trans* fatty acids (as elaidic acid) of fat and oil samples [two solid-state (margarine and ghee) and two liquids-state (olive oil and corn oil)]. Results showed that elaidic acid content in margarine was not affected by heat at 120 °C at all studied heating durations. At 150, 190 and 250 °C, there is a cubic significant relationship between elaidic acid content and time of heating. Elaidic acid content in ghee was not affected by heat treatment at 120 °C, while at 150 °C, there was a cubic significant relationship between elaidic acid content and heating time. At 190 °C, there was a negative linear relationship between elaidic acid content and time of frying, while at 250 °C, there was oscillatory relationship in the amounts of elaidic acid with time of heating. As a conclusion, all margarine and ghee samples analyzed in this study had elaidic acid before and after heat treatment used in the study. However, corn oil and olive oil were free from elaidic acid before and after studied heat treatments. Therefore, it is recommended to cook and bake with vegetable oils (such as corn oil) instead of solid fats, and to keep margarine and ghee consumption as low as possible in nutrition.

Keywords: *trans* fatty acid, elaidic acid, margarine, ghee, corn oil, olive oil, HPLC, frying time

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1. Introduction

Trans fatty acid has been a matter for debate in last years, especially after the studies which showed that industrial *trans* acids such as elaidic acid occurs in hydrogenation of vegetable oils, margarine, fat spread, shortening vegetable oils and food containing hydrogenation of plant oils and margarine, such as bakery, sweets, cakes, donuts, and frying foods, made with partially-hydrogenated fats [1,2,3]. The human lipase enzyme digest, transport, and process dietary lipids such as triglycerides, fats, and oils and works only on the *cis* configuration and cannot metabolize *trans* fatty acids [1,4]. Consumption of diets high in hydrogenated fat and/or *trans* fatty acids has been shown to have an adverse effect on lipoprotein profiles with respect to cardiovascular disease risk [3,5,6,7].

Trans fatty acids are defined as the geometrical isomers of monounsaturated and polyunsaturated fatty acids having non-conjugated [interrupted by at least one methylene group (-CH₂-)] carbon-carbon double bonds in the *trans* configuration [8]. This includes the *trans* monoenes

mainly stereoisomers of elaidic acid, and the *trans* isomers of polyunsaturated fatty acids (*trans* dienes, *trans* trienes) with non-conjugated carbon-carbon double bonds, produced through hydrogenation of oils and fats (both vegetable and animal/marine origin) in the presence of a suitable chemical catalyst [2]. The definition, however, excludes conjugated *trans* fatty acids present naturally in animal fats and their products that include conjugated linoleic acid. The US FDA defined *trans* fatty acids as "unsaturated fatty acids that contain one or more isolated (i.e. non-conjugated) double bonds in a *trans* configuration" [9].

There are many factors that affect formation of *trans* fatty acid including partial hydrogenation, refining process, baking, and frying [3,10]. These factors may change a double bond from a *cis* position to a *trans* position (geometric isomerization) or move to another position in the carbon chain (positional isomerization) and both types of isomerization may occur in the same molecule [11]. Different *trans* isomers can be formed depending on difference factors affecting their production [12].

Analytical methods used to determine the *trans* fatty acid and/or especially octadecenoic acid 18:1 (elaidic acid) isomers carried out according to official methods reported by the American Oil Chemists' Society (AOCS) and the

Association of Official Analytical Chemists (AOAC) are HPLC, GC, and IR [9,10]. Nine official methods are used to determine *trans* fatty acid in food [13], each of these methods has advantages and drawbacks. Therefore, the objective of this work is to study the effect of heating on selected types of fat and oil (margarine, ghee, olive oil, and corn oil) on the formation of elaidic acid by using HPLC-UV method.

2. Materials and Methods

2.1. Fat and Oils

Margarine (solid fat) used in this study (BESLER Grad veKimya San, Turkey) contained fat (60%), water, emulsifier (mono and diglyceride soya lecithin, salt (0.4 %), preservative (potassium sorbate), acidity regulator (citric acid), vitamins (A and D), color (Beta-carotene), and flavors. Pure vegetable ghee (solid fat) (AL-GHZAL, vegetable oils industries Co. Ltd, Palestine), contained refined palm oil, vitamins [A (24 I.U/gm), D (3 I.U/gm)], beta-carotene, and flavors. Corn Oil (obtained from local market in Palestine). Extra virgin olive oil was obtained from olive trees grown in Nuba, Hebron - Palestine. The fats and oils under investigation were analyzed for their contents of *trans* fatty acids (elaidic acid) before subjecting them to the treatments under study. Corn oil and olive oil samples were free from elaidic acid while margarine and ghee contained 0.89 ± 0.32 and $0.84 \pm 0.25\%$ (w/w) elaidic acid respectively.

2.2. Chemicals and Reagents

Acetonitrile HPLC grade was obtained from J.T Baker (NJ, USA). Acetic acid and n-hexane were obtained from Merck (Darmstadt, Germany).

2.3. Frying Process and Heating Treatments

Three liters of each fats or oils were placed into fryer (Horng Yun Steel Factory, Yon Lin, Taiwan), and 1 kg tube slices potato (2 cm diameter x 4 cm long) were fried at 4 different temperatures (120, 150, 190 and 250 °C) for different times (10, 30, 60 and 180 min) in each type of fat or oil separately.

2.4. HPLC conditions

Mobile phase was prepared by mixing 800 ml acetonitrile (HPLC grade) with 200 ml purified water and 1.0 ml of glacial acetic acid was added. The mobile phase was filtered using 0.45 µm membrane filter and degassed by sonication, to avoid column blockage by any particulate matters and to prolong pump and column life. The mobile phase was then left few minutes to reach room temperature. The column used for separation was C₁₈ (150 mm long x 4.0 mm inner diameter) with particle size of 5 µm. The flow rate was 2 ml/ minute and injection volume was 50 µl. The temperature of auto-sampler was 15 °C, while the temperature of column was 25 °C. The wavelength of UV detector was 205 nm, and the run time was 30 min.

2.5. Preparation of Standard and Sample Solutions

Oil and fat samples were prepared by dissolving 1 g of each fat and oil in 50 ml of n-hexane then was shaken well and filtered by using 0.45 µm membrane filter. Standard solution of oleic and elaidic acid was prepared by dissolving 10 mg of each standard in 100 ml n-hexane to obtain a solution with 100 ppm concentration of both oleic and elaidic acid.

2.6. Statistical Analysis

Scatter plots and mean plots were induced between % elaidic and each independent variable of temperature and time of frying, visual inspection of plots suggest relations between the % elaidic and the independent variables. Ridge regression analysis was used to examine the relationships between % elaidic and the studied factors (temperature and time of frying). One Way Analysis of Variance (ANOVA) with Tukey HSD Multiple Comparisons Post Hoc tests were used to test if there are differences in % elaidic acid means due to the time of heating and temperature. Analyses were performed by using the Statistical Package for Social Science (SPSS) version 18 on Windows version 7 [SPSS Inc., USA].

3 Results and Discussion

3.1. Verification of Standard Solution

Peak identification of oleic acid and elaidic acid was based mainly on their retention times, where the retention times for oleic and elaidic acid standards were found to be 14.8 and 17.6 min, respectively with good separation of the two peaks (Figure 1).

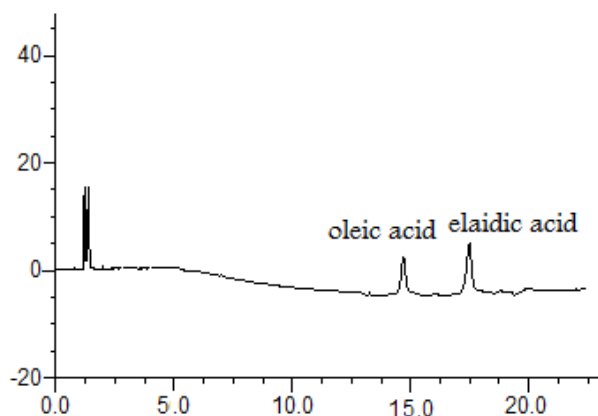


Figure 1. Chromatogram of oleic and elaidic acid with a concentration of 10 mg L^{-1} of each. HPLC conditions: C₁₈ column (15 cm and 4.0 mm inner diameter, particle size of 5 µm). Mobile phase is acetonitrile: water (80:20, V/V) containing 0.1% acetic acid. Flow rate: 2.0 ml min^{-1} , injection volume: 50 µL. Column: C₁₈, 5 µm, 15 cm length, 4.6 mm inner diameter, UV detection: 205 nm

3.2. Elaidic Acid Formation in Margarine

The percentage of elaidic acid in margarine heated to different temperatures was determined and presented in Figure 2. Results showed that the amount of elaidic acid

vary with temperature. According to statistical results, elaidic acid content in margarine heated at 120°C didn't change at all time intervals. This indicates that the structural (positional) isomerization and geometrical isomerization in which the *cis* isomers of fatty acids in margarine is stable and not affected by applied heat treatment at 120°C. It was reported previously that *cis* isomerization to *trans* may occur at 150°C [14]. The results are in accordance with these results where it was found that the amount of elaidic acid in margarine heated at 150, 190 and 250°C varies with time in which there is a cubic nonlinear significant relationship between percentage of elaidic acid and time of heating.

At 150°C, the percentage of elaidic acid decreased at 10 and 30 min, then increased as time of heating increased. At 190°C, the percentage of elaidic acid increased at 10, 30 and 60 min, but at 180 min the percentage of elaidic acid remained constant. At 250°C, the highest percentage of elaidic acid was recorded at 10 min and the lowest percentage was detected at 30 min, however after 30 min the percentage of elaidic acid increased when time of heating increased.

After 10 minutes of heating, the results showed that the lowest percentage of elaidic acid was recorded at 190°C, and the highest percentage of elaidic was recorded at 250°C. At 30 and 60 min, the lowest percentage of elaidic acid was recorded at 250°C, and the highest percentage of elaidic acid was found at 120°C. After 180 min for all applied heat treatments, the results revealed that the percentage of elaidic acid went to the approximate values, which is almost to the extent of the value of elaidic acid in the original margarine without heat treatment. The highest influence affected of elaidic acid was recorded at 250°C.

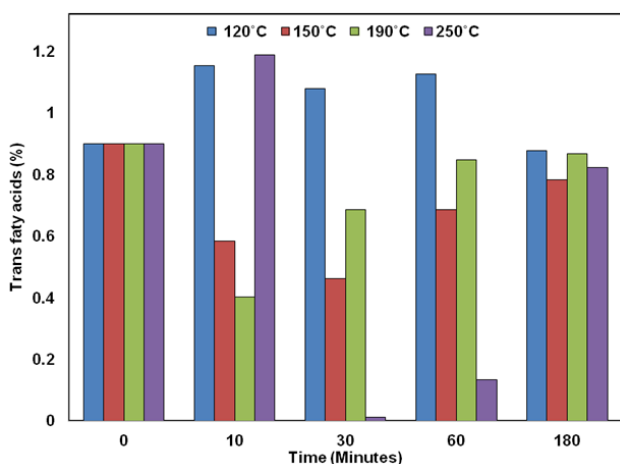


Figure 2. Effect of heating on the percentage of elaidic acid of margarine samples treated at four different temperatures (120, 150, 190 and 250°C)

The dependence of *trans* fatty acid content of margarine on heating temperature is in agreement with other investigators [15] who showed that the content of *trans* fatty acids increased with time and with temperature and the rate of *cis-trans* isomerisation and polymerization depends on the temperature according to Arrhenius equation. Other researchers reported an insignificant increase in the amount of *trans* isomers at extreme temperatures during baking [16]. *Trans* fatty acids produced during heat treatments depend on temperature and length of treatment [17]. Thermal treatments of fats

and oils such as deodorization, cooking, and frying, generate *trans* fatty acids isomers with limited double bond migration along the carbon chain. Unlike partial hydrogenation, heating induces the formation of mainly *trans* 18:2 and *trans*-18:3 [18].

3.3. Elaidic Acid Formation in Ghee

Figure 3 shows the influence of heat treatment at 4 temperatures (120, 150, 190 and 250°C) in different heating durations (10, 30, 60 and 180 min) on the percentage of elaidic acid in ghee samples. It was found that the highest percentage of elaidic acid was recorded at 150°C for 60 min, while the lowest percentage of elaidic was found at 250°C for 30 min.

According to statistical analyses, the obtained result at 120°C showed no significant difference in the percentage of elaidic acid in ghee treated at 120°C at different time intervals. At 150°C, there was a cubic significant relationship between percentage of elaidic and time of heating. At 190°C, there is a negative linear relationship between the percentage of elaidic acid and time of frying. At 190°C, the percentage of elaidic acid decrease when the time of heating increase. At 250°C, the highest percentage of elaidic acid occurred at 60 min, where the lowest percentage of elaidic acid occurred at 30min.

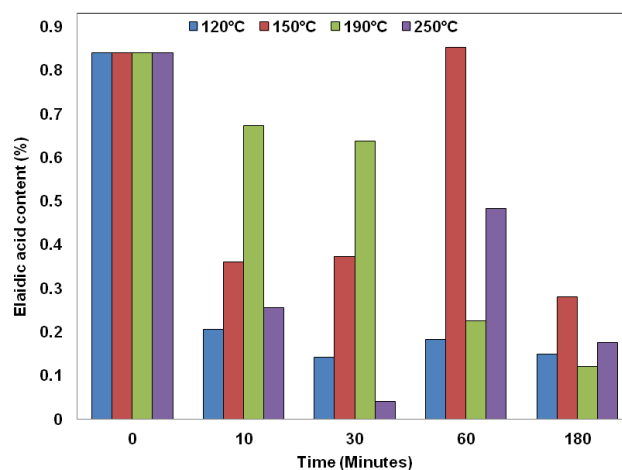


Figure 3. Effect of heating (temperature degree and heating duration) on the percentage of elaidic acid in ghee samples treated at four different temperatures (120, 150, 190 and 250°C)

3.4. Elaidic acid formation in liquid oils

The effect of heat treatment on the formation of elaidic acid in two liquid oils (olive and corn oil) was investigated in this study. Results showed that elaidic acid was not detected in samples treated with four different heat temperatures (120,150,190 and 250°C) and different heating time intervals (10, 30, 60 and 180 min) for each heat treatment and in each oil type. Some workers found that among cooking methods, only stir-frying increased *trans* fat in corn oil whereas baking, and pan-frying procedures did not make changes in *trans* fat content as compared to untreated corn oils [19]. Other investigators documented that the *trans* formation from *cis* configuration in unsaturated lipids is inevitable steps during autoxidation [20,21]. Which implies that heat treatment in both olive

oil and corn oil conducted in this study did not reach oxidation of olive oil and corn oil.

4. Conclusions and Recommendations

All of margarine and ghee samples had elaidic acid before and after heat treatment. Margarine has higher elaidic acid percentage than ghee, while olive oil and corn oil do not have elaidic acid before and after heat treatments. It is recommended therefore to issue standards to deal with the amount of *trans* fats occurring with margarine and ghee. It is recommended also for fat and oil factories to modify processing methods of margarine to decrease the amount of *trans* fatty acids. For the consumers, it is recommended to keep margarine consumption as low as possible by limiting foods that contain them.

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Competing Interests

None declared.

Abbreviations

US FDA- United States Food and Drug Administration,
AOCS- the American Oil Chemists' Society,
AOAC- the Association of Official Analytical Chemists,
HPLC- High Pressure Liquid Chromatography,
GC- Gas Chromatography,
IR- Infra Red,
UV- Ultra Violet,
ANOVA- Analysis of Variance,
C18- Carbon 18.

References

- [1] Eckel, R., Borra, S., Lichtenstein, A.H., and Yin-Piazza, S.Y., "Understanding the complexity of trans fatty acid reduction in the American diet", *American Heart Association Trans Fat Conference, Circulation* 2007, 115. 2231-2246. 2007.
- [2] Martin, C.A., Milinsk, M.C., Visentainer, J.V., Matsushita, M., and De-Souza, N.E., "Trans fatty acid-forming processes in foods: a review", *Anais da Academia Brasileira de Ciências*, 79 (2). 343-350. 2007.
- [3] Ganguly, R., and Pierce G.N., "The toxicity of dietary trans fat", *Food and Chemical Toxicology*, 78. 170-176. 2015.
- [4] Brundiek, H.B., Evitt, A.S., Kourist, R., and Bornscheuer, U.T., "Creation of a lipase highly selective for trans Fatty acids by Protein Engineering", *Angewandte Chemie* 51 (2). 412-414, 2012.
- [5] Lopez-Garcia, E., Schulze, M.B., Meigs, J.B., Manson, J.E., Rifai, N., Stampfer, M.J., Willett, W.C., and Hu, F.B., "Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction", *Journal of Nutrition*, 135. 562-566. 2005.
- [6] Iqbal, M.P., "Trans fatty acids - a risk factor for cardiovascular disease", *Pakistan Journal of Medical Science*, 30 (1). 194-197. 2014.
- [7] Gebauer, S.K., Chardigny, J.M., Jakobsen, M.U., Lamarche, B., Lock, A.L., Proctor, S.D., and Baer, D.J., "Effects of Ruminant trans Fatty Acids on Cardiovascular Disease and Cancer: A Comprehensive Review of Epidemiological, Clinical, and Mechanistic Studies", *Advances in Nutrition*, (2). 232-354. 2011.
- [8] Codex Alimentarius. (2012). <http://www.codexalimentarius.org> [accessed on 25 September 2017].
- [9] Stolyhwo, A., and Rutkowska, J., "An improved silver ion HPLC combined with capillary gas chromatography of cis/trans fatty acids in alimentary fats and human milk fat", *Food Analytical Methods*, 6. 457-469. 2013.
- [10] Narkwichian, N., "Analysis of trans fatty acid in some foods by attenuated total reflection-fourier transform infrared spectroscopy", *Master's Thesis, Chulalongkorn University, Thailand*. 2009.
- [11] Shabbir, M.A., Dilber, A., Raza, A., Suleria, H.A.R., Saeed, M., and Sultan, S., "Influence of thermal processing on the formation of trans fats in various edible oils", *Journal of Food Processing and Preservation*, 39 (6). 1475-1484. 2015.
- [12] Marais, C.D.W., "The determination of cis and trans fatty acid isomers in partially hydrogenated plant oils", *M.Sc. Thesis, University of Stellenbosch, Stellenbosch, South Africa*, 2007.
- [13] Juan áda, P., Ledoux, M., and S õedio, J.L., "Analytical methods for determination of trans fatty acid content in food", *European Journal of Lipid Science and Technology*, 109 (9). 901-917. 2007.
- [14] Li, C., Zhang, Y., Li, S., Wang, G., Xu, C., Deng, Y., and Wang, S., "Mechanism of formation of trans fatty acids under heating conditions in triolein", *Journal of Agricultural and Food Chemistry*, 61 (43). 10392-10397. 2013.
- [15] Cihelkova, K., Zarubova, M., Hradkova, I., Filip V., and Šmidrkal, J., "Changes of sunflower oil polyenoic fatty acids under high temperatures", *Czech Journal of Food Science*, 27. 13-16. 2009.
- [16] Przybylski, R., and Aladedunye, F., "The formation of trans fats during food preparation", *Canadian Journal of Dietetic Practice and Research*, 73 (2). 98-101. 2012.
- [17] Liu, W.H., Inbaraj, B.S., and Chen B.H., "Analysis and formation of trans fatty acids in hydrogenated soybean oil during heating", *Food Chemistry*, 104. 1740-1749. 2007.
- [18] Ledoux, M., Juan áda, P., and S õedio, J.L., "Trans fatty acids: definition and occurrence in foods", *European Journal of Lipid Science and Technology*, 109, 891-900. 2007.
- [19] Song, J., Park, J., Jung, J., Lee, C., Gim, S.Y., Ka, H., Yi, B., Kim, M.J., Kim, C.I., and Lee, J., "Analysis of trans fat in edible oils with cooking process", *Toxicology Research*, 31 (3). 307-312. 2015.
- [20] Choe, E., and Minutes, D.B., "Mechanisms and factors for edible oil oxidation", *Comprehensive reviews in Food Science and Food Safety*, 5 (4). 169-186. 2006.
- [21] Talpur, M.Y., Hassan, S.S., Mahesar, S.A., Kandhro, A.A., Kara, H., Sherazi, S.T.H., and Channa, A., "Rapid determination of trans fats in thermally oxidized soybean oil by transmission FT-IR spectroscopy", *American Journal of Food Science and Technology*, 3 (1). 19-23. 2015.