Processing induced trans fatty acid quantification analysis of selected edible oils and their probable outcome

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The repeated processing and over-cooking of food may adversely affect the oil quality, causing various physiological and chemical changes in it. Isomerization of the double bond is one such process which may generate trans fatty acids (TFAs) in food. In the present study, we analysed the effect of thermal processing (frying, heating and microwave treatment) on trans fat formation in selected oils/fats with reference to their fatty acids bond length and double-bond position. It was observed that frying and microwave treatment significantly affected the TFA content in oils/fats.

Keywords: Cooking oil, frying, isomerization, microwave treatment, thermal processing, trans fatty acids.

A healthy body is necessary for our well-being and in this regard, consuming the right quality of oil/fat in our diet is equally important. While cooking food, repeated processing and over-cooking may generate various chemical and physiological changes in the oil. Isomerization of the double bond is one process that forms trans fatty acids (TFAs) in food. These trans fats are the geometrical isomers of unsaturated fatty acids with a minimum of one non-conjugated, C=C bond in the trans configuration[1,2]. The trans configuration affects the functional and physio-chemical properties of these fatty acids (FAs), gradually affecting their metabolism in the body[3]. Therefore, the World Health Organization, Geneva, Switzerland, has recommended a limited intake of trans fats in the diet (less than 1% of total calorie in diet)[4]. It is assumed that many domestic or commercial processing treatments may provide the required activation energy for bond isomerization, hence generating unhealthy TFAs[5]. In this study, we analysed the effect of thermal processing (frying, heating and microwave treatment) on the TFA content of selected edible oils/fats generally consumed in India. The study also provides a comparative analysis of the trans fat formation in selected oils/fats with reference to their fatty acids bond length and double-bond position.

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Materials and methods

Five major oils/fats consumed in India, viz. mustard oil, palm oil, soybean oil, hydrogenated fat (vanaspati) and desi ghee (clarified butter) were selected for the study. These oils/fats have different degrees of unsaturation (double bonds), thereby affecting the isomerization of fatty acids differently. The treatments studied were as follows:

(i) Frying (first, second, third and fourth frying cycles): During each frying cycle, 100 g potato fingers was fried in 1 l oil samples at 200°–220°C till golden brown (10 min approximately). For the second and third frying cycles, the same oil was procured after frying fresh potato fingers four and six times respectively[6,7].

(ii) Heating (100°C, 200°C, 300°C and 400°C): Six hundred millilitres of oil was taken in a 1 l beaker and heated on a thermostat hot plate with continuous shaking at 100°C, 200°C, 300°C and 400°C for 1 h (with ±5°C variation)[8,9].

(iii) Microwave treatment: Two hundred and fifty millilitres of each oil sample was treated in a microwave oven at 2450 MHz and 150 W power for 10, 20, 30 and 40 min (refs 10, 11).

Reagents and standards

The five FAs and fatty acid methyl ester (FAME) standards, viz. palmitelaidic acid (PA), elaidic acid (EA), vaccenic acid (VA), linoleic acid (LA) isomer mix and linolenic acid (LLA) isomer mix were purchased from Sigma-Aldrich (India) (purity; ≥99.99%).

Sample preparation

In 0.2 g of oil sample, approximately 10 ml of methanolic NaOH (1 N) was added and refluxed for 10 min. Further, 5 ml of 14% methanolic boron trifluoride was added and refluxed again for 2 min. Then 5 ml *n*-heptane was added and the sample was cooled. Thereafter, conc. NaCl was added to separate the organic layer (centrifuged for better

Table 1. Changes in trans fatty acid (TFA) composition of selected oils/fats during frying

Sample	Linoleic acid methyl ester (%)	Linolenic acid methyl ester (%)	Elaidic acid methyl ester (%)	Vaccenic acid methyl ester (%)	Palmitelaidic acid methyl ester (%)	Total trans fat percentage in the product
Soybean oil – control	0.14 ± 0.02	0.32 ± 0.04	0.15 ± 0.08	ND	ND	0.61 ± 0.010[a]
Soybean oil – first frying	0.18 ± 0.07	0.53 ± 0.05	0.16 ± 0.02	ND	0.01 ± 0.01	0.88 ± 0.11[b]
Soybean oil – second frying	0.49 ± 0.09	0.55 ± 0.07	0.21 ± 0.04	ND	ND	1.26 ± 0.19[c]
Soybean oil – third frying	0.72 ± 0.14	0.65 ± 0.08	0.30 ± 0.08	ND	ND	1.57 ± 0.35[cd]
Soybean oil – fourth frying	0.83 ± 0.06	0.73 ± 0.05	0.37 ± 0.11	ND	0.05 ± 0.01	1.87 ± 0.31[d]
Mustard oil – control	0.13 ± 0.06	0.39 ± 0.08	0.33 ± 0.10	ND	0.03 ± 0.01	0.80 ± 0.16[a]
Mustard oil – first frying	0.25 ± 0.04	0.47 ± 0.17	0.42 ± 0.06	ND	0.04 ± 0.01	1.17 ± 0.28[b]
Mustard oil – second frying	0.34 ± 0.06	0.53 ± 0.11	0.45 ± 0.02	ND	0.07 ± 0.03	1.38 ± 0.26[c]
Mustard oil – third frying	0.40 ± 0.08	0.59 ± 0.02	0.47 ± 0.07	ND	0.07 ± 0.03	1.52 ± 0.38[cd]
Mustard oil – fourth frying	0.49 ± 0.11	0.67 ± 0.13	0.52 ± 0.05	0.02 ± 0.01	0.08 ± 0.02	1.74 ± 0.46[d]
Palm oil – control	0.20 ± 0.04	0.15 ± 0.06	0.45 ± 0.12	ND	0.13 ± 0.05	0.93 ± 0.23[a]
Palm oil – first frying	0.28 ± 0.07	0.22 ± 0.04	0.50 ± 0.14	ND	0.17 ± 0.09	1.15 ± 0.25[a]
Palm oil – second frying	0.37 ± 0.09	0.31 ± 0.09	0.56 ± 0.07	ND	0.19 ± 0.07	1.41 ± 0.36[ab]
Palm oil – third frying	0.52 ± 0.08	0.47 ± 0.11	0.63 ± 0.08	0.03 ± 0.01	0.27 ± 0.05	1.9 ± 0.27[b]
Palm oil – fourth frying	0.60 ± 0.08	0.58 ± 0.07	0.72 ± 0.11	0.04 ± 0.02	0.34 ± 0.05	2.18 ± 0.32[c]
Hydrogenated fat – control	1.68 ± 0.20	1.37 ± 0.11	6.13 ± 0.36	ND	0.24 ± 0.08	9.42 ± 0.33[a]
Hydrogenated fat – first frying	1.87 ± 0.16	1.42 ± 0.27	6.42 ± 0.51	ND	0.42 ± 0.07	10.13 ± 0.37[b]
Hydrogenated fat – second frying	2.21 ± 0.30	1.84 ± 0.61	7.27 ± 1.02	ND	0.56 ± 0.11	11.88 ± 0.62[bc]
Hydrogenated fat – third frying	2.57 ± 0.24	2.11 ± 0.14	7.91 ± 0.81	ND	0.58 ± 0.07	13.17 ± 0.62[cd]
Hydrogenated fat – fourth frying	3.25 ± 0.18	2.72 ± 0.21	8.05 ± 0.82	ND	0.62 ± 0.06	14.64 ± 0.99[d]
Clarified butter – control	0.41 ± 0.06	2.68 ± 0.13	ND	0.10 ± 0.02	ND	3.19 ± 0.33[a]
Clarified butter – first frying	0.56 ± 0.11	2.92 ± 0.11	ND	0.16 ± 0.03	ND	3.64 ± 0.32[b]
Clarified butter – second frying	0.74 ± 0.14	3.16 ± 0.15	0.02 ± 0.01	0.20 ± 0.03	ND	4.11 ± 0.38[bc]
Clarified butter – third frying	1.03 ± 0.16	3.52 ± 0.16	0.05 ± 0.02	0.34 ± 0.05	ND	4.94 ± 0.31[d]
Clarified butter – fourth frying	0.91 ± 0.08	3.29 ± 0.12	0.04 ± 0.02	0.28 ± 0.07	ND	4.52 ± 0.24[c]

All data are expressed as mean ± SD ($n = 3$). Values with different superscripts in the same column differ significantly ($P \leq 0.05$). ND, Not determined.

separation). Then 1.0 ml of the top layer was transferred into a glass tube and diluted to 10 ml with *n*-heptane[12].

GC analysis of FAME

GC Clarus 500 chromatograph from Perkin Elmer with flame ionization detector (FID) was utilized to analyse fatty acids using a fused silica capillary column SP-2560 (length 30 m and internal diameter 320 μm). High-purity nitrogen was used as the carrier gas with a flow rate of 1 ml/min, and zero air and hydrogen were used as fuel gas with a flow rate of 450 and 45 ml/min respectively. The oven temperature was programmed at 130°C for 4 min, increased at the rate of 2.5°C/min up to 240°C, and then ramped at the rate of 5.0°C up to 260°C for 20 min. The detector and injector temperatures were 280°C and 220°C respectively. The injection volume was 2 μl in split-less mode[13].

Calculation and statistical analysis

The individual FAs were estimated from their corresponding peak areas and the total TFA was determined by adding individual FAs. Results obtained were analysed using one-way ANOVA followed by Dunnett's post hoc test; $P < 0.05$ was considered a significant value.

Results and discussion

During domestic and commercial cooking, we often heat the oil for a prolonged period on a low flame to obtain crisp or microwave our food for repeated heating which may generate harmful TFAs in it. The present study examined the effect of various thermal treatments on the quality of oil, which may adversely affect our health. Table 1 gives the TFA content of all five selected edible oils/fats during different thermal processing treatments.

Effect of frying treatment on TFA content in selected oils

A clear increase in TFAs was observed during the frying cycles (Table 1). The trans fat isomer found in palm oil and hydrogenated fat was EA (C18 : 1, t), while in soybean oil, mustard oil and clarified butter, the predominant TFA was LLA. Mustard oil also contained a good amount of EA.

Out of the five samples, maximum isomerization was identified in hydrogenated fat, followed by clarified butter. Soybean oil (Figure 1), palm oil and mustard oil also significantly increased trans fat content. The results also indicated a significant increase in LA isomers in the oil samples during the frying cycles ($P < 0.05$).

Table 2. Changes in TFA composition of selected oils/fats during heat treatment

Sample	Linoleic acid methyl ester (%)	Linolenic acid methyl ester (%)	Elaidic acid methyl ester (%)	Vaccenic acid methyl ester (%)	Palmitelaidic acid methyl ester (%)	Trans fat percentage in the product
Soybean oil – control	0.14 ± 0.02	0.34 ± 0.04	0.15 ± 0.08	ND	ND	0.63 ± 0.08[a]
Soybean oil – 100°C	0.16 ± 0.03	0.32 ± 0.06	0.17 ± 0.04	ND	ND	0.65 ± 0.09[a]
Soybean oil – 200°C	0.19 ± 0.06	0.34 ± 0.07	0.18 ± 0.04	ND	0.01 ± 0.01	0.71 ± 0.08[ab]
Soybean oil – 300°C	0.23 ± 0.08	0.36 ± 0.08	0.18 ± 0.02	ND	ND	0.77 ± 0.11[b]
Soybean oil – 400°C	0.32 ± 0.06	0.41 ± 0.05	0.22 ± 0.11	ND	0.03 ± 0.01	0.98 ± 0.13[c]
Mustard oil – control	0.12 ± 0.06	0.37 ± 0.08	0.32 ± 0.10	ND	0.02 ± 0.01	0.80 ± 0.11[a]
Mustard oil – 100°C	0.12 ± 0.04	0.35 ± 0.17	0.32 ± 0.06	ND	0.02 ± 0.01	0.80 ± 0.06[a]
Mustard oil – 200°C	0.14 ± 0.06	0.38 ± 0.11	0.30 ± 0.02	ND	0.03 ± 0.03	0.84 ± 0.11[ab]
Mustard oil – 300°C	0.16 ± 0.08	0.42 ± 0.02	0.31 ± 0.07	ND	0.03 ± 0.03	0.92 ± 0.13[b]
Mustard oil – 400°C	0.19 ± 0.11	0.47 ± 0.13	0.33 ± 0.05	0.03 ± 0.01	0.06 ± 0.02	1.08 ± 0.11[c]
Palm oil – control	0.20 ± 0.04	0.15 ± 0.06	0.45 ± 0.12	ND	0.13 ± 0.05	0.93 ± 0.10[a]
Palm oil – 100°C	0.21 ± 0.04	0.17 ± 0.06	0.42 ± 0.12	ND	0.13 ± 0.05	0.93 ± 0.12[a]
Palm oil – 200°C	0.22 ± 0.09	0.19 ± 0.09	0.45 ± 0.07	ND	0.12 ± 0.07	0.98 ± 0.10[b]
Palm oil – 300°C	0.27 ± 0.08	0.25 ± 0.11	0.48 ± 0.08	0.03 ± 0.01	0.19 ± 0.05	1.21 ± 0.20[c]
Palm oil – 400°C	0.30 ± 0.08	0.29 ± 0.07	0.51 ± 0.11	0.04 ± 0.02	0.23 ± 0.05	1.36 ± 0.18[d]
Hydrogenated fat – control	1.68 ± 0.24	1.37 ± 0.11	6.13 ± 0.36	ND	0.24 ± 0.08	9.42 ± 0.73[a]
Hydrogenated fat – 100°C	1.65 ± 0.20	1.39 ± 0.15	6.16 ± 0.26	ND	0.22 ± 0.06	9.40 ± 0.57[a]
Hydrogenated fat – 200°C	1.87 ± 0.30	1.43 ± 0.61	6.38 ± 1.02	0.03 ± 0.02	0.39 ± 0.11	10.10 ± 0.89[b]
Hydrogenated fat – 300°C	2.52 ± 0.24	1.83 ± 0.14	6.80 ± 0.81	0.16 ± 0.04	0.54 ± 0.07	11.85 ± 0.85[c]
Hydrogenated fat – 400°C	3.05 ± 0.18	2.35 ± 0.21	7.13 ± 0.82	0.18 ± 0.04	0.61 ± 0.06	13.31 ± 1.06[cd]
Clarified butter – control	0.41 ± 0.06	2.68 ± 0.13	ND	0.10 ± 0.02	ND	3.19 ± 0.43[a]
Clarified butter – 100°C	0.44 ± 0.06	2.65 ± 0.13	ND	0.13 ± 0.02	ND	3.21 ± 0.53[a]
Clarified butter – 200°C	0.58 ± 0.14	2.98 ± 0.15	0.02 ± 0.01	0.31 ± 0.03	ND	3.89 ± 0.48[b]
Clarified butter – 300°C	0.92 ± 0.16	3.21 ± 0.16	0.05 ± 0.02	0.38 ± 0.04	ND	4.56 ± 0.31[c]
Clarified butter – 400°C	0.81 ± 0.08	3.09 ± 0.12	0.04 ± 0.02	0.30 ± 0.03	ND	4.24 ± 0.22[bc]

All data are expressed as mean ± SD (n = 3). Values with different superscripts in the same column differ significantly ($P \leq 0.05$).

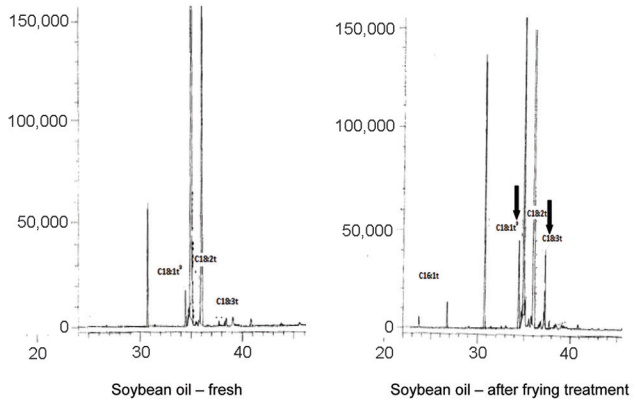

Figure 1. Gas chromatography (GC) analysis of soybean oil before and after frying (fourth frying) treatment.

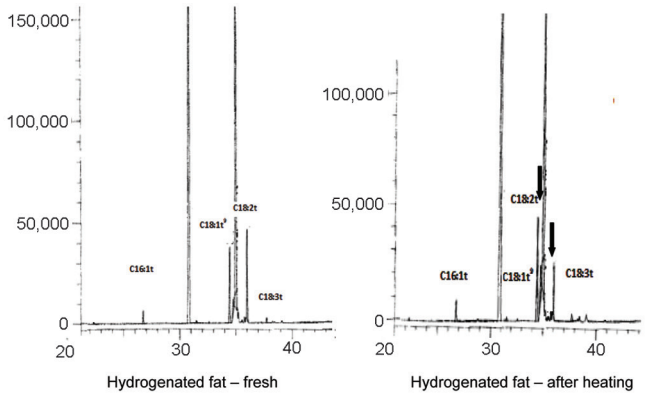

Figure 2. GC analysis of hydrogenated fat before and after heat (400ºC) treatment.

Effect of heat treatment on TFA content

No change was observed at 100°C and 200°C temperature; however, at 300°C and 400°C significant increase in TFA was observed (Table 2). In hydrogenated fat and soybean oil, a maximum increase was observed for trans LA content, whereas mustard oil and clarified butter showed a significant increase in LLA content (Figures 2 and 3). In palm oil, trans LLA and PA content increased more significantly.

Effect of microwave treatment on TFA content in selected oils

During each microwave treatment, a significant increase in TFA was observed in all the samples (Figures 4 and 5). The distribution of trans LA C18.2 was found highest followed by trans LLA C18.1 and the least for EA (Table 3). However, comparative TFA profiles of the oil samples showed that the microwave treatments had a marginal impact on their fatty acids composition.

Table 3. Changes in TFA composition of soybean oil during microwave treatment

Sample	Linoleic acid methyl ester (%)	Linolenic acid methyl ester (%)	Elaidic acid methyl ester (%)	Vaccenic acid methyl ester (%)	Palmitelaidic acid methyl ester (%)	Total trans fat percentage in the product
Soybean oil – control	0.14 ± 0.02	0.34 ± 0.04	0.15 ± 0.08	ND	ND	0.63 ± 0.12[a]
Soybean oil – 10 min	0.18 ± 0.07	0.40 ± 0.05	0.17 ± 0.02	ND	0.01 ± 0.01	0.76 ± 0.086[b]
Soybean oil – 20 min	0.21 ± 0.09	0.43 ± 0.07	0.19 ± 0.04	ND	ND	0.83 ± 0.11[bc]
Soybean oil – 30 min	0.24 ± 0.14	0.48 ± 0.08	0.23 ± 0.08	ND	ND	0.95 ± 0.23[c]
Soybean oil – 40 min	0.31 ± 0.06	0.57 ± 0.05	0.26 ± 0.11	ND	0.04 ± 0.01	1.18 ± 0.28[cd]
Mustard oil – control	0.13 ± 0.06	0.39 ± 0.08	0.33 ± 0.10	ND	0.03 ± 0.01	0.80 ± 0.15[a]
Mustard oil – 10 min	0.17 ± 0.04	0.45 ± 0.17	0.36 ± 0.06	ND	0.04 ± 0.01	0.96 ± 0.08[b]
Mustard oil – 20 min	0.19 ± 0.06	0.51 ± 0.11	0.39 ± 0.02	ND	0.03 ± 0.02	1.05 ± 0.12[c]
Mustard oil – 30 min	0.20 ± 0.08	0.55 ± 0.02	0.41 ± 0.07	ND	0.03 ± 0.01	1.12 ± 0.20[cd]
Mustard oil – 40 min	0.24 ± 0.11	0.60 ± 0.13	0.44 ± 0.05	0.02 ± 0.01	0.04 ± 0.02	1.28 ± 0.28[d]
Palm oil – control	0.20 ± 0.04	0.15 ± 0.06	0.45 ± 0.12	ND	0.13 ± 0.05	0.93 ± 0.11[a]
Palm oil – 10 min	0.24 ± 0.07	0.17 ± 0.04	0.50 ± 0.14	ND	0.16 ± 0.09	1.07 ± 0.11[b]
Palm oil – 20 min	0.27 ± 0.09	0.19 ± 0.09	0.54 ± 0.07	ND	0.14 ± 0.07	1.14 ± 0.14[c]
Palm oil – 30 min	0.29 ± 0.08	0.19 ± 0.11	0.58 ± 0.08	ND	0.16 ± 0.05	1.22 ± 0.18[d]
Palm oil – 40 min	0.34 ± 0.08	0.22 ± 0.07	0.66 ± 0.11	0.04 ± 0.02	0.18 ± 0.05	1.44 ± 0.15[e]
Hydrogenated fat – control	1.68 ± 0.24	1.37 ± 0.11	6.13 ± 0.36	ND	0.24 ± 0.08	9.57 ± 0.84[a]
Hydrogenated fat – 10 min	1.77 ± 0.16	1.59 ± 0.27	6.32 ± 0.51	ND	0.27 ± 0.07	9.95 ± 0.72[ab]
Hydrogenated fat – 20 min	1.84 ± 0.30	1.81 ± 0.61	6.67 ± 1.02	ND	0.36 ± 0.11	10.68 ± 0.48[b]
Hydrogenated fat – 30 min	2.03 ± 0.24	2.00 ± 0.14	6.81 ± 0.81	ND	0.42 ± 0.07	11.26 ± 0.97[cd]
Hydrogenated fat – 40 min	2.21 ± 0.18	2.18 ± 0.21	7.05 ± 0.82	ND	0.51 ± 0.06	11.95 ± 1.06[d]
Clarified butter – control	0.41 ± 0.06	2.68 ± 0.13	ND	0.10 ± 0.02	ND	2.93 ± 0.77[a]
Clarified butter – 10 min	0.50 ± 0.11	2.77 ± 0.11	ND	0.18 ± 0.03	ND	3.45 ± 0.52[b]
Clarified butter – 20 min	0.71 ± 0.14	3.02 ± 0.15	0.03 ± 0.01	0.21 ± 0.03	ND	3.96 ± 0.41[c]
Clarified butter – 30 min	0.78 ± 0.16	3.12 ± 0.16	0.05 ± 0.02	0.22 ± 0.05	ND	4.17 ± 0.31[d]
Clarified butter – 40 min	0.73 ± 0.08	3.05 ± 0.12	0.01 ± 0.02	0.19 ± 0.06	ND	3.98 ± 0.38[c]

All data are expressed as mean ± SD (n = 3). Values with different superscripts in the same column differ significantly ($P \leq 0.05$).

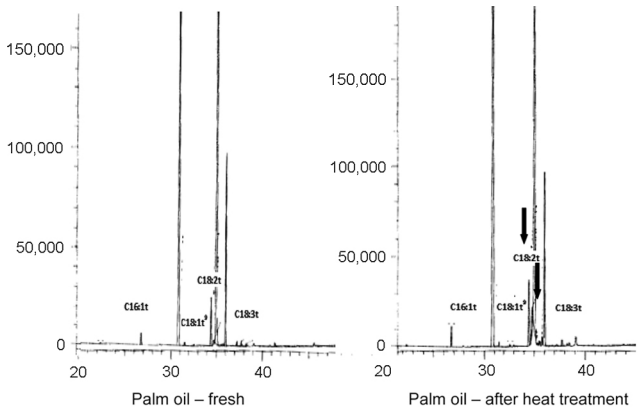

Figure 3. GC analysis of palm oil before and after heat (300ºC) treatment.

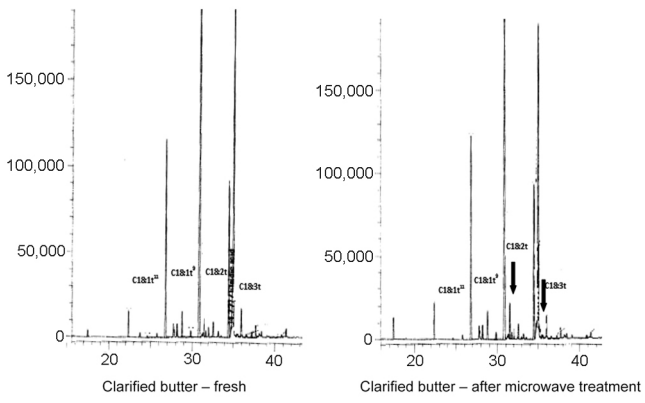

Figure 4. GC analysis of clarified butter before and after microwave (40 min) treatment.

Repetitive use of oil at high temperatures in the presence of moisture and air causes thermal degradation of the oil[7]. Both high temperature and longer frying time increased the amount of trans isomers during frying. In a recent study, Jain and Jain[14] reported that TFA content in the oil increases with increasing progressive frying cycles and temperature. Generally, deep fat frying is carried out at high temperatures in the presence of moisture and air. These frying oils undergo physical and chemical deterioration (hydrolysis, polymerization and oxidation). It also results in the formation of high molecular weight polar compounds, which may affect the frying performance and storage stability of the fried products[15]. Similarly, during the heating of oils, fatty acid moiety in the glyceride molecules can undergo several changes like isomerization, oxidation, intra-cyclization and degradation into several secondary products. The results obtained after heat treatment were similar to those of Afaneh *et al.*[16], where an increase in EA was observed in treated oil samples on heating above 150°C. However, below this temperature no significant change was observed. The changes in the TFA content of different oil samples demonstrated that the higher the temperature, the faster is the degradation of

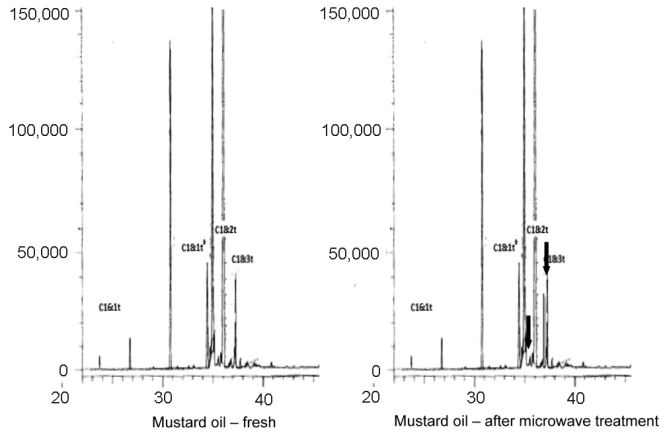

Figure 5. GC analysis of mustard oil before and after microwave treatment.

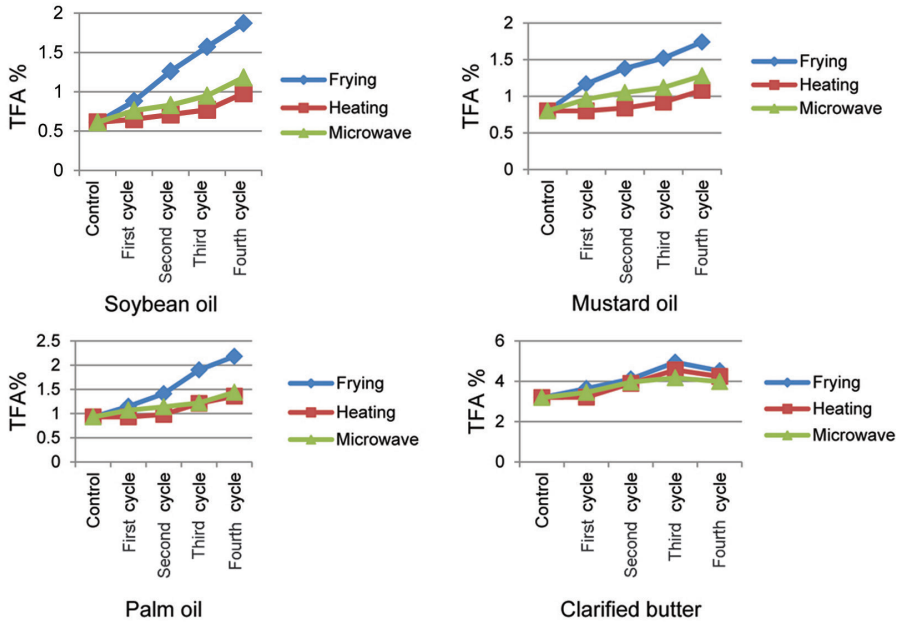

Figure 6. Increase in trans fatty acid (TFA) content in selected oils during thermal processing.

cis fatty acids (Table 2). The results of this study proved that cis fatty acid could undergo degradation under severe heating conditions, and the concentration of the degraded products depended on heating temperature and time. A similar study dealing with the heating of sunflower oil at 220°C, 240°C and 270°C for 5 h reported an increase in TFA by 3% and 11% at 240°C and 270°C respectively[17]. Also, no TFA formation was observed by Mollenken[18] in several vegetable oils heated at 170°C and 350°C for 30 min or 200°C and 220°C for 16 h. It was thus concluded that TFAs are formed under severe cooking conditions.

Kemeny *et al.*[19] studied the degree of isomerization (DI) of individual fatty acids during heat treatment and concluded that in oils tested, DI C18 : 2 was significantly higher than DI C18 : 1. Wolff[20] studied the formation of LLA geometrical isomers in linseed oil when it was heated under vacuum at different temperatures (190–260°C) for a long duration (2–16 h).

Heating food in a microwave oven is caused by the interaction of an electromagnetic field with the chemical constituents of food[21]. Fats have a great capacity for storing microwave energy, although they have a small dielectric loss. Various studies have shown that microwave heating has some degradative effects on the quality and composition of oils. However, Osawa and Gonçalves[22] reported small changes in the fatty acid profile and low formation of trans isomers in the cottonseed oil used for frying chicken in a microwave oven. Ali *et al.*[23] also reported that longer microwave heating times resulted in a greater degree of oil deterioration.

The activation energy for polyunsaturated fatty acids isomerization decreases when the number of cis double

Table 4. Comparing the effect of different processing treatments on trans fatty acid content

Sample	Fresh oil	Frying				Heating (°C)				Microwave (min)			
		First cycle	Second cycle	Third cycle	Fourth cycle	100	200	300	400	10	20	30	40
Soybean oil	0.61 ± 0.12^{a}	0.88 ± 0.11^{b}	1.26 ± 0.19^{c}	1.57 ± 0.31^{cd}	1.87 ± 0.35^{d}	0.65 ± 0.09^{a}	0.71 ± 0.12^{ab}	0.77 ± 0.10^{b}	0.98 ± 0.16^{c}	0.76 ± 0.86^{b}	0.83 ± 0.11^{bc}	0.95 ± 0.16^{c}	1.18 ± 0.20^{cd}
Mustard oil	0.80 ± 0.16^{a}	1.17 ± 0.18^{b}	1.38 ± 0.26^{c}	1.52 ± 0.28^{cd}	1.74 ± 0.26^{de}	0.80 ± 0.06^{a}	0.84 ± 0.12^{ab}	0.92 ± 0.17^{b}	1.08 ± 0.14^{c}	0.96 ± 0.08^{b}	1.05 ± 0.12^{c}	1.12 ± 0.18^{cd}	1.28 ± 0.23^{d}
Palm oil	0.93 ± 0.13^{a}	1.15 ± 0.25^{a}	1.41 ± 0.26^{ab}	1.9 ± 0.22^{b}	2.18 ± 0.32^{c}	0.93 ± 0.12^{a}	0.98 ± 0.10^{b}	1.21 ± 0.16^{c}	1.36 ± 0.19^{d}	1.07 ± 0.11^{b}	1.14 ± 0.14^{c}	1.22 ± 0.14^{d}	1.44 ± 0.15^{e}
Hydrogenated fat	9.42 ± 0.33^{a}	10.13 ± 0.57^{b}	11.88 ± 0.42^{bc}	13.17 ± 0.52^{cd}	14.64 ± 0.69^{d}	9.40 ± 0.47^{a}	10.10 ± 0.59^{b}	11.85 ± 0.65^{c}	13.31 ± 1.02^{cd}	9.95 ± 0.72^{ab}	10.68 ± 0.48^{b}	11.26 ± 0.97^{cd}	11.95 ± 1.06^{d}
Clarified butter	3.19 ± 0.23^{a}	3.64 ± 0.32^{b}	4.11 ± 0.28^{bc}	4.94 ± 0.31^{d}	4.52 ± 0.24^{c}	3.21 ± 0.28^{a}	3.89 ± 0.31^{b}	4.56 ± 0.31^{c}	4.24 ± 0.22^{bc}	3.45 ± 0.22^{b}	3.96 ± 0.31^{c}	4.17 ± 0.28^{d}	3.98 ± 0.31^{c}

All data are expressed as mean ± SD (n = 3). Values with different superscripts in the same column differ significantly ($P \leq 0.05$).

bonds increases; hence the LLA trans isomers dominate in trans fat[24]. Figure 6 shows a comparative increase in TFA content in selected oils during different thermal treatments. Among the treatments, the maximum increase was observed during frying, followed by microwave and heat treatment (Table 4). This may be because during frying and microwave treatment, the polar–nonpolar transition and dipole interaction provide nucleation for the isomerization reaction. However, during heating the required activation energy could not have been reached at lower temperatures.

More recently, concurrent with domestic and industrial food practices, there has been not only an increase in fat consumption by individuals but also a shift in the quality of the fat consumed[25]. This shift can be correlated with the increased prevalence of lifestyle diseases such as cardiovascular disease, diabetes and immune dysfunction, leading to poor health outcomes. Hence it is advised to avoid repeated cooking/processing of food in the same oil. We must also avoid repeated heating of food in the microwave, which may isomerize the fat into TFAs and lead to various metabolic disorders.

Conclusion

Trans fats found in processed foods are a cheap and effective way to extend their shelf life. While trans fats have been beneficial to food manufacturers, they are considered harmful to humans and can cause cardiovascular disease, diabetes, cancer and other related disorders. In 2015, the United States Food and Drug Administration (FDA) reported that partially hydrogenated oils are unsafe for human consumption. Therefore, it is important to eliminate trans fats in our diet. The present study reveals that various thermal processing treatments produce TFAs and their formation has been linked to temperature, time and the number of processing cycles. Hence to maintain a safe level of TFA in our diet, we should be careful while selecting cooking oil. We must also avoid repeated processing/cooking of oil or oil-rich foods that may generate TFAs.

1. Codex, A., Guidelines on nutrition labelling, CAC/GL 2-1985; www.codexalimentarius.org/input/download/standards/34/CXG_0-02e.pdf
2. Kodali, D. R., Trans fats – chemistry, occurrence, functional need in foods and potential solutions. In *Trans Fats Alternatives* (eds Kodali, D. R. and List, G. R.), AOCS Press, Champaign, Illinois, USA, 2005, pp. 1–25.
3. Mozaffarian, D., Jacobson, M. F. and Greenstein, J. S., Food reformulations to reduce trans fatty acids. *N. Eng. J. Med.*, 2010, **362**, 2037–2039.
4. Diet, nutrition and the prevention of chronic diseases. WHO/FAO Technical Report Series, 2003, No. 916, p. 160.
5. Changmo, L., Yubin, Z. and Shuai, L., Mechanism of formation of trans fatty acids under heating conditions in triolein. *J. Agric. Food Chem*., 2013, **61**, 10392–10397.
6. Jun, C. H., Lian, Z. J. and Chun, W. Z., Effect of frying on trans fatty acid formation in soybean oil. *Eur. J. Lipid Sci. Technol.*, 2012, **114**, 287–293.
7. Talpur, M. Y., Sherazi, S. T. H., Mahesar, S. A., Bhanger, M. I. and Kandhro, A., Consequence of commercial fish frying on some quality parameters of oil with special reference to trans fat. *Int. J. Food Prop.*, 2011, **14**(5), 1124–1135.
8. Li, A., Yiming, H., Wang, F. and Li, W., Determination of thermally induced soybean oil by attenuated total reflectance Fourier transform infrared spectroscopy and gas chromatography analysis. *J. Agric. Food Chem.*, 2012, **60**, 10709–10713.
9. Tsuzuki, W., Matsuoka, A. and Kaori, U., Formation of trans fatty acids in edible oils during the frying and heating process. *Food Chem.*, 2010, **123**, 976–982.
10. Emma, C., Maria, T. R., Elena, V. and Nicoletta, P., Microwave heating of different vegetable oils: relation between chemical and thermal parameters. *LWT – Food Sci. Technol.*, 2010, **43**, 1104–1112.
11. Olabemiwo, O. M., Esan, A. O., Adepoju, A. J. and Omodara, N. B., Effect of microwave heating on fatty acid profiles of three Nigerian vegetable oils. *IOSR J. Appl. Chem.*, 2014, **7**(4), 51–54.
12. Fatty acid in oils and fats preparation of methyl ester, boron trifluoride method. In AOAC Official Methods of Analysis, 2002, 17th edn, pp. 400–405.
13. Fat (total, saturated, unsaturated, and monosaturated) in cereal products: acid hydrolysis capillary gas chromatographic. In AOAC Official Methods of Analysis, 2002, 17th edn, pp. 384–386.
14. Jain, A. and Jain, S., Effect of frying temperature/frying cycles on trans fat content of groundnut oil. *Plant Arch*., 2020, **20**, 2565–2568.
15. Abiona, O. O., Awojide, S. H., Anifowoshe, A. J. and Babalola, O. B., Comparative study on effect of frying process on the fatty acid profile of vegetable oil and palm oil. *E-Int. Sci. Res. J.*, 2011, **3**, 321–328.
16. Afaneh, I., Abbadi, J., Rimawi, F. A., Dabbas, G. A. and Sawalha, S., Effect of frying temperature and duration on the formation of trans fatty acids in selected fats and oils. *Am. J. Food Sci. Technol.*, 2017, **5**, 245–248.
17. Kamel, B. and Kakuda, Y., *Technological Advances in Improved and Alternative Sources of Lipids*, Chapman & Hall, UK, 2004, 20th edn, pp. 296–303.
18. Mollenken, H., Trans fatty acids in heated hemp seed oil. *J. Int. Hemp Assoc.*, 2008, **5**, 21–25.
19. Kemeny, Z., Recseg, K., Henon, G., Kovari, K. and Zwobada, F., Deodorization of vegetable oils: prediction of trans polyunsaturated fatty acid content. *J. Am. Oil Chem. Soc.*, 2001, **78**, 973–979.
20. Wolff, R. L., Trans isomers of α-linolenic acid in deodorized oils. *Lipid Technol*., 1997, **4**, 36–39.
21. Yoshida, H., Hirakawa, Y. and Abe, S., Influence of microwave roasting on positional distribution of fatty acids of triacylglycerols and phospholipids in sunflower seeds. *Eur. J Lipid Sci. Technol.*, 2001, **4**, 201–207.
22. Osawa, C. C. and Gonçalves, L. A. G., Changes in breaded chicken and oil degradation during discontinuous frying with cottonseed oil. *Cienc. Tecnol. Aliment. Campinas*, 2012, **4**, 692–700.
23. Ali, M. A., Nouruddeen, Z. B., Muhamad, I. I., Latip, R. A. and Othman, N. H., Effect of microwave heating on oxidative degradation of sunflower oil in the presence of palm olein. *Sains Malays.*, 2014, **43**(8), 1189–1195.
24. Maga, J. A., Twomey, J. A. and Cohen, M., Effect of baking methods on the fatty acid composition of potatoes. *J. Food Sci.*, 2009, **42**, 1669–1670.
25. Cordain, L., Eaton, S. B., Sebastian, A., Mann, N. and Lindeberg, S., Origin and evolution of western diet: health implications for the 21st century. *Am. J. Clin. Nutr.*, 2005, **2**, 341–354.

Received 18 February 2021; revised accepted 20 August 2022

doi: 10.18520/cs/v123/i12/1455-1461