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Evaluating composition of fatty acids in commonly used edible oils and fats on repeated frying for its nutritional quality implications

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ORIGINAL ARTICLE

Abstract

Continuous frying of many food items in the same vegetable oils or partially hydrogenated vegetable oils (fats) by homemakers and commercial vendors has become a routine practice in many developing countries of Asia. The present study evaluated the composition of fatty acids (FAs) in commonly used edible oils (canola oil, sunflower oil, corn oil, and linseed oil) and fats (Utility ghee, Kisan ghee, Shama ghee, and Dalda ghee). Further, it quantified changes in the composition of their FAs, especially regarding transformation of cis-FAs to trans FAs, during repeated frying for 0, 1, 3, 6, 12, and 24 h at 180°C. A large variability ($p < 0.001$) was observed in composition of FAs among unheated fats and oils. Compared to oils, the fats had higher ($p < 0.05$) contents of saturated FAs (SFAs) and trans FAs, and lower ($p < 0.05$) contents of cis-unsaturated FAs (cis-UFAs). Repeated frying for 24 h consistently decreased ($p < 0.05$) the contents of cis-UFAs and increased ($p < 0.05$) the contents of SFAs and trans FAs in all fats and oils. The SFA–cis-UFA ratio of all fats and oils increased ($p < 0.05$) during 24 h of heating, reflecting deterioration in their nutritional quality. In conclusion, repeated frying of fats and oils induces cis–trans conversion and increases SFA–cis-UFA ratio.

Keywords: cis-fatty acids; fats; frying; hydrogenation; oils; trans fatty acids

Introduction

Edible vegetable oils and fats are rich source of energy, essential fatty acids (FAs), fat soluble vitamins, and many other health-beneficial compounds, and as such they are an indispensable part of a healthy human diet (Cui *et al.,* 2017; Eratte *et al.,* 2017; Tian *et al.*, 2023). However, there is increasing evidence that inappropriate consumption of oils and fats are associated with increased development of many life-style diseases, such as obesity, type-2 diabetes, coronary heart diseases, and cancer (Chen *et al.,* 2016; Cui *et al.,* 2017; Venkata and Subramanyam, 2016).

Globally, frying of foods in heated oil/fat is a popular method of food preparation. In South Asia, deep frying is extensively used by homemakers and commercial vendors to produce desirable flavor, aroma, color (golden brown), and texture (crispiness) in a variety of food items (Bhardwaj *et al.,* 2016). Moreover, it is also a quick method of food preparation. However, owing to high costs of edible oils and fats, the same oil/fat is repeatedly used for frying of food items both at home and commercially. This practice is particularly prevalent in developing countries, such as Pakistan, where economic constraints drive the reuse of frying oils and fats, potentially compromising their nutritional quality. However, repeated heating of oils and fats at high temperatures (180–190°C) during deep frying, particularly for extended period of time, predisposes cis-unsaturated fatty acids (cis-UFAs) into thermal oxidation, isomerization, and polymerization (Li *et al.,* 2023; Liang *et al.,* 2023; Zhang *et al.,* 2012), leading to partial transformation of cis-UFAs into saturated fatty acids (SFAs) and trans fatty acids (trans FAs) (Cui *et al.,* 2017; Tsuzuki *et al.,* 2010; Zhuang *et al.*, 2022).

Production of hydrogenated fats, commonly known as ghee, involves partial hydrogenation of vegetable oils, such as soybean, canola, sunflower, peanut, and palm oils. This process, carried out under controlled temperature and pressure, converts liquid oils into semi-solid fats, improving oxidative and thermal stability while extending their shelf life. However, it may also generate trans FAs, which have a non-conjugated trans-configuration. In contrast, butter is the fat extracted from animal milk. Butter is a natural product and does not undergo industrial hydrogenation. Research has established that continuous consumption of SFAs, particularly non-conjugated trans FAs, increases the level of low-density lipoprotein and insulin resistance, and elevate the risks of lifestyle diseases, such as type-2 diabetes (Bhardwaj *et al.*, 2016; Micha and Mozaffarian, 2009), cardiovascular diseases (Chen *et al.*, 2016; Yanai *et al.,* 2015), and cancer (Venkata and Subramanyam, 2016).

Normally, cis-UFAs in edible oils have many benefits for human health, as all carbon–carbon double bonds are of cis-configuration. On the other hand, trans FAs are unsaturated FAs with non-conjugated carbon–carbon double bond(s) in trans-configuration and are produced in edible oils/fats because of partial hydrogenation of cis-UFAs during industrial processing as well as during cooking at high temperatures for extended period, such as deep frying (Tsuzuki *et al.,* 2010). The heat-induced changes in the composition of FAs and formation of trans FAs not only depends on the heating temperature but also on the duration of heating and on the cis-UFAs contents of oils and fats (Bhardwaj *et al.,* 2016). For example, the rate and extent of hydrogenation and oxidation increases with the increasing degree of unsaturation of FAs in oils/fats (Khan *et al.,* 2009, 2011). On the other hand, partial hydrogenation of vegetable oils enhances thermal and oxidative stability, which, in turn, increases the shelf life and stability during deep frying (Walter and Mozaffarian, 2007). There is a possibility that highly beneficial oils with high level of cis-polyunsaturated fatty acids (cis-PUFAs) are more damaged by frying than the less beneficial saturated fats. Therefore, knowledge about the effect of frying frequency and duration on the changes in the composition of FAs and formation of trans FAs in different oils and fats commonly used for frying of food items must be enhanced.

In many developing countries, such as Pakistan, different types of vegetable oils and fats are repeatedly used for food frying. However, insufficient information is available on their FAs composition and transformation during frying and cooking, making it difficult for consumers to select stable and nutritionally beneficial oil(s) and fat(s) for traditional cooking and frying of food. The present study is therefore designed to evaluate and study changes in the FAs composition of commonly used oils and fats in Pakistan, especially in terms of SFAs–cis-UFAs ratio and cis- to trans-isomerization of cis-UFAs during different frying durations.

Materials

Chemicals and reagents

External standard for FAs' peaks identification, Supelco 37 Component FAME Mix, was purchased from Supelco (Poole, Dorset, UK). Standard for C18:1 trans-11, C18:2 cis-9, trans-11, and C18:2 trans-10, cis-12 was purchased from Larodan Fine Chemicals (AB, Malmo, Sweden). All the chemicals used for analysis were of analytical grade. Sodium hydroxide (purity > 99%), sulfuric acid (purity: 70%), and hydrochloric acid (purity: 38%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (GC grade, purity: 99.9%) and hexane (GC grade, purity: ≥98.5%) were supplied by Merck (Darmstadt, Germany). Three replicate samples of commercially available, freshly supplied partially hydrogenated oils (fats), namely Kisan ghee (Faisalabad Oil Refinery Pvt. Ltd., Pakistan), Shama ghee (Shama Ghee Mill, Nowshera, Pakistan), Dalda ghee (Dalda Foods Ltd., Karachi, Pakistan), and Utility ghee (Utility Stores Corporation Pvt., Pakistan) and freshly supplied oils—canola oil, sunflower oil, corn oil, and linseed oil—were bought from three local markets and stored at 4°C until heat treatments. The four ghee brands evaluated were primarily produced from a blend of palm, soybean, and canola oils, subjected to partial hydrogenation at 180–200°C with nickel catalysts to achieve a semi-solid texture. Shama ghee is subjected to more extensive hydrogenation, resulting in higher trans FA content.

Methods

Heat treatments of ghee and oils

Before heat treatment, sub-samples in triplicate were collected from the selected brands of fats and oils for FAs analysis. To study the stability of fats and oils during deep frying, 450 g of each sample was taken in a steel vessel and heated at 180°C (to mimic frying temperature) on digital hot plate, with temperature accuracy of ±0.5°C. First subsample (10 mL) was collected from each vessel after 1 h of heating. The subsample was immediately stored in a freezer and the remaining fat and oil samples in the vessels were refrigerated overnight (ca. 12 h); these were heated again on next day at 180°C for 2 h. The next sampling was conducted (a total of 3-h heating) and stored in a freezer. After second sampling, the remaining fats/oils in vessels were refrigerated overnight (ca. 12 h). Similar procedure was followed for 6-, 12-, and 24-h heating, with a 12-h cooling period coming up after every sampling point. The temperature of oil/ghee sample(s) was continuously measured at every 30 min to ensure that it was maintained at 180°C. Samples in triplicate from each treatment were collected along with the control sample that had not undergone any heat treatment.

Transmethylation of fatty acids

By using a transesterification process that Khan *et al.* (2009) have described earlier, the triglycerides were transformed into fatty acid methyl esters (FAMEs). In all, 3 mL of 0.5-M NaOH methanol were used to dissolve 0.2-mL subsample. After 10 min of vortexing, the mixture was baked at 50°C for 30 min. Following 2–3 min of cooling, 2 mL of HCl/methanol (1:1 v/v) was added. The mixture was vortexed and baked for 10 min at 50°C. Then, 2 mL of hexane and 2 mL of water were added. After another vortex, the mixture was centrifuged for 5 min at 800×*g*. The fraction of hexane was collected. Extra hexane was added to the residual aqueous fraction to achieve greater recoveries. The mixture was vortexed, and the hexane fraction was collected after it was centrifuged for 5 min at 800×*g*. After being pooled, the hexane extract was chilled and dried. After redissolving in 1 mL of hexane, residues were put into gas chromatography (GC) vials.

Gas chromatography

As described previously (Khan *et al.*, 2012), FAMEs were measured using gas chromatography (GC; TRACE GC Ultra; Thermo Electron Corp., Waltham, MA, USA) fitted with an autosampler and a flame-ionization detector (FID). In short, methylated FAs were separated at a constant flow rate of 1.5 mL/min using a fused silica capillary column filled with hydrogen as a carrier gas. A GC was used to inject 1 μL of material at a split ratio of 1:50.

For GC, the following program was used: a starting temperature of 140°C was kept for 4 min, after which it was raised to 240°C at a rate of 4°C per minute and held for an additional 20 min. The injector and FID had temperatures of 250°C and 280°C, respectively. Peaks were discovered by comparing retention time to equivalent peaks of external standards. Fatty acids C18:1 trans-11; C18:1 trans-10; C18:1 trans-9; C18:1 trans-6+7+8, C18:2 trans-11, and cis-15 were recognized with the help of elution sequence reported by Loor *et al.* (2004) and Shingfield *et al.* (2006). Using the internal standard's peak area as a reference, the individual FAME contents were computed from the peak area.

Statistical analysis

The composition of FAs of unheated oils and fats was analyzed using PROC GLM procedure of statistical analysis system (SAS; SAS Institute, NC, USA) using the following model (1):

$$Yij = \mu + FOi + eij, \qquad (1)$$

where Yij is a dependent variable under examination, μ is the general mean, FOi the effect of oil/fat type, and eij is the residual. The effect of heating duration on the composition of FAs of oils and fats was analyzed with repeated measure ANOVA using PROC MIXED procedure of SAS (Littell *et al.*, 2006). The following model (2) was used:

$$Yijk = \mu + OFTi + Fdj + OFTi \times Fdj + eijk, \qquad (2)$$

where Yijk is a dependent variable, μ is the general mean, OFTi is the fixed effect of oils or fat type (i = Utility ghee, Kisan ghee, Shama ghee, or Dalda ghee, or i = canola oil, sunflower oil, corn oil, or linseed oil), Fdj is the fixed effect of the repeated measures of frying duration (J = 1, 3, 6, 12, and 24 h), OFTi × Fdj is the fixed effect of the interaction of oil/fat type and heating duration, and eijk is the residual. Different covariance structures of repeated matrices were evaluated using the Akaike information criterion and the Schwarz Bayesian criterion according to Littell *et al.* (1998) and Wang and Goonewardene (2004). The ANTE (1) covariance structure was used in the models. Pair-wise difference at $p < 0.05$ among mean values was computed using the Tukey–Kramer test adjusted for multiple comparisons of least square means.

Results

Fatty acid profile of unheated edible fats and oils

The FA profiles of selected commercial brands of partially hydrogenated edible fats and oils are given in Table 1. There was large variability ($p < 0.001$) in the contents of all major FAs, SFAs, total cis-UFAs, and total cis-PUFAs among selected fats and oils. Compared to oils, fats had higher ($p < 0.05$) content of SFAs (78.6–88.1 vs. 34.3–50.7 g/100 g) and lower ($p < 0.05$) content of cis-UFAs (6.65–13.8 vs. 29.7–55.9 g/100 g). Unheated oils had lower ($p < 0.05$) SFAs–cis-UFA ratios (0.09:0.17) than unheated fats (0.61:1.71).

In fats, the major SFAs were C16:0 and C18:0, ranging from 30.1–46.7 and 2.85–3.55 g/100 g, respectively, while the major cis-UFAs were C18:1n-9c and C18:2n-6c, ranging from 26.2–47.1 and 2.89–10.5 g/100 g, respectively. Unheated fats contained lower ($p < 0.05$) amount of health-beneficial cis-PUFAs (2.91–10.9 g/100 g) and high ($p < 0.05$) amount of unhealthy trans FAs (C18:1n-9t, 0.18–12.81 g/100 g), compared to unheated oils. Comparison of FA composition across fat brands revealed that Shama ghee had the highest ($p < 0.05$) content of less-healthy C16:0 (46.7 g/100 g) and unhealthy C18:1n-9t (12.81 g/100 g), and the lowest ($p < 0.05$) content of beneficial cis-UFAs (29.7 g/100 g), while Kisan ghee had the lowest ($p < 0.05$) content of less-healthy C16:0 (30.1 g/100 g) and the highest ($p < 0.05$) content of beneficial cis-UFAs (55.9 g/100 g). In oils, the predominant cis-UFAs were C18:1n-9 (16.6–72.9 g/100 g), C18:2n-6 (8.33–21.5 g/100 g), and C18:3n-3 (0.05–53.7 g/100 g), and the major SFA was C16:0 (4.72–10.3 g/100 g). However, there was large variation ($p < 0.001$) in the composition of FAs in oils, with linseed oil having the maximum ($p < 0.05$) level of C18:3n-3 (53.7 g/100 g) and total PUFAs (62.0 g/100 g), while corn oil had the maximum ($p < 0.05$) level of cis-UFAs (88.1 g/100 g).

Effect of heating duration on fatty acids profile of fats and oils

Extended (re)heating for 24 h markedly altered ($p < 0.001$) the contents of all major individual FAs, SFAs,

Table 1. Comparison of fatty acid (FA) composition (g/100 g total FA) of raw (unheated) vegetable oils and commercially hydrogenated vegetable oils (fats) used for frying.

	C14:0	C16:0	C18:0	C18:1n-9c	C18:1n-9t	C18:2n-6c	C18:3n-3c	SFAs	Total Cis-UFA	Total Cis-PUFA
Fats										
Shama ghee	1.38^{c}	46.7^{a}	3.55^{a}	26.2^{g}	12.8^{a}	2.89^{g}	0.05^{e}	51.7^{a}	29.7^{f}	2.91^{f}
Kisan ghee	0.72^{d}	30.1^{b}	3.55^{a}	47.1^{d}	0.39^{e}	8.15^{f}	0.72^{d}	34.3^{b}	55.9^{d}	8.87^{e}
Utility ghee	1.49^{a}	46.2^{a}	2.85^{c}	37.6^{e}	1.51^{b}	8.53^{e}	$0.26^{d,e}$	50.6^{a}	45.1^{e}	8.82^{e}
Dalda ghee	1.43^{b}	46.4^{a}	3.28^{b}	36.1^{f}	0.18^{f}	10.5^{a}	$0.34^{d,e}$	51.2^{a}	46.9^{e}	10.9^{d}
Oils										
Canola oil	0.11^{e}	6.64^{d}	2.65^{d}	66.3^{b}	0.10^{f}	12.5^{b}	3.26^{c}	9.29^{d}	82.0^{b}	15.7^{c}
Corn oil	0.11^{e}	6.51^{d}	2.56^{e}	62.8^{c}	0.53^{d}	21.5^{a}	3.80^{b}	9.08^{d}	88.1^{a}	25.2^{b}
Sunflower oil	ND	10.3^{c}	2.80^{c}	72.9^{a}	1.11^{c}	10.0^{c}	$0.46^{d,e}$	13.8^{c}	83.3^{b}	10.4^{d}
Linseed oil	ND	4.72^{e}	1.76^{f}	16.6^{h}	0.00^{f}	$8.33^{e,f}$	53.7^{a}	6.65^{e}	78.6^{c}	62.0^{a}
SEM	0.005	0.176	0.017	0.285	0.026	0.065	0.197	0.356	0.368	0.141
p value	***	***	***	***	***	***	***	***	***	***

Notes: PROC GLM procedure of Statistical Analysis System (SAS; SAS Institute, NC) was used to test differences in fatty acid composition of unheated oil and ghee.
Mean values in the same column with different superscript letters differ at $p < 0.05$.
$^{***}p < 0.001$. ND: not determined.
SEM: standard error of mean; C14:0: myristic acid; C16:0: palmitic acid; C18:0: stearic acid; C18:1n-9c: oleic acid; C18:2n-6c: linoleic acid; C18:3n-3c: linolenic acid; total cis-UFA: total unsaturated fatty acids with cis-configuration (C18:1n-9c + C18:2n-6c + C18:3n-3c); total SFA, total saturated fatty acid (C14:0 + C16:0 + C18:0); total cis-PUFA: total polyunsaturated fatty acids with cis-configuration (C18:2n-6c + C18:3n-3c).

total cis-UFAs, and total cis-PUFAs in the selected four brands of edible oils (Table 2). Irrespective of oil-type, the SFAs and trans FAs increased ($p < 0.001$) during the 24-h frying process, while cis-UFAs and cis-PUFAs decreased ($p < 0.001$). The SFAs–cis-UFA ratio is an important index of oil/fat nutritional quality, with a relatively lower ratios reflecting higher quality.

The SFA–cis-UFA ratio of all fats increased during 24-h heating. With respect to individual FAs, there was a marked increase ($p < 0.05$) in the content of C16:0 in canola oil (6.64–19.3 g/100 g), corn oil (6.51–34.6 g/100 g), sunflower oil (10.3–28.0 g/100 g), and linseed oil (4.72–30.6 g/100 g), while the content of 18:1n-9c markedly decreased ($p < 0.05$) in canola oil (66.3–48.0 g/100 g), corn oil (62.8–45.2 g/100 g), and sunflower oil (72.9–52.5 g/100 g). In linseed oil, however, there was a marked decrease ($p < 0.05$) in the content of 18:3n-3c (53.0–20.0 g/100 g). C18:1n-9t was the major trans FA accumulated during 24-h heating; however, there was substantial variation in the accumulation of C18:1n-9t in canola oil (0.10–5.42 g/100 g), corn oil (0.43–3.21 g/100 g), sunflower oil (1.11–4.55 g/100 g), and linseed oil (0.00–5.72 g/100 g). Table 3 presents data on the effects of (re)heating for 24 h on changes in the content of major individual FAs, total SFAs, total cis-UFAs, and total cis-PUFAs in the four brands of fats. Unheated fats already had higher contents of C16:0, SFAs, and C18:1n-9t; however, the heating induced more or less similar changes in their FAs composition as observed for the selected oils. The content of SFAs and trans FAs increased ($p < 0.001$), while the content of cis-UFAs and cis-PUFAs decreased ($p < 0.001$) during the 24-h frying, irrespective of the fat-type. Except Kisan ghee, the unheated fats contained a very high content (>45 g/100 g) of unhealthy C16:0, which further increased (>55 g/100 g) after 24-h heating. Among the fats, Kisan ghee contained relatively lower proportion (30.1 g/100 g) of C16:0; however, it increased to 50 g/100 g after 24-h heating. Despite a wide range, the SFA–cis-UFA ratio of all fats increased during 24-h heating. The unheated Shama ghee had a higher ($p < 0.05$) content of C18:1n-9t (12.0 g/100 g), which increased to 16.6 g/100 g after 24-h heating. Despite lower ($p < 0.05$) contents of C18:1n-9t before heating, there was significant accumulation of C18:1n-9t during 24-h frying with Kisan ghee (0.36–2.10 g/100 g), Utility ghee (1.51–5.65 g/100 g), and Dalda ghee (1.51–5.65 g/100 g).

Discussion

The results present the first comprehensive dataset on the composition of FAs of commonly used edible fats and oils in Pakistan. The study also provides novel data on the changes in the composition of FAs and accumulation of trans FAs during extended frying (24 h), reflecting common deep-frying practices in South Asia. Owing to consumer preferences for crispy/roasted texture, fried aroma and pleasant (golden to brown) color, frying is widely used for preparing a variety of foods and snacks at homes, restaurants, bakeries, and (semi)-commercial levels in Pakistan, similar to in many other Asian countries (Bhardwaj *et al.*, 2016; Cui *et al.*, 2017; Siddiq *et al.*, 2018; Tsuzuki *et al.,* 2010). For economic reasons, the same oil/fat is often repeatedly used for frying over extended periods at home and the food services industry. Our results revealed that despite large variation in the composition of FAs of unheated fats and oils, repeated frying markedly decreased the contents health-beneficial cis-UFAs and cis-PUFAs and increased the contents of less-healthy SFAs and unhealthy trans FA in all fats and oils, highlighting the need for regular assessment of the quality of frying fats/oils to avoid long-term deleterious effects on human health.

Partially hydrogenated edible fats are often preferred for frying at home and commercial level because of their low cost, longer shelf life, and desirable aroma, taste, and texture of fried foods (Agrawal *et al.,* 2008; Bhardwaj *et al.,* 2016; Pattanayak, 2019). As such fats are consumed in significant amounts, contributing an average of 42–43% of daily energy intake by humans. All the fats evaluated in the present study contained a high proportion of SFAs (30.1–46.4 g/100 g) and trans FAs (0.18–12.8 g/100 g), with lower proportion of cis-UFAs (29.7–55.9 g/100 g), even prior to frying (Table 1), making their continuous consumption a long-term risk for human health (Bhardwaj *et al.,* 2011; Yanai *et al.,* 2015). In agreement with our findings, previous studies have reported high contents of SFAs (41 g/100 g) (Bhardwaj *et al.,* 2016) and trans FAs (up to 40 g/100 g of total FAs) (Destaillats and Angers, 2002; Pattanayak, 2019) in commercially hydrogenated edible fats.

Our results are also consistent with the findings of Szabo *et al.* (2022), who observed significant increase in SFAs and trans FAs in plant-based cooking oils after 10 cycles of 5 min heating, particularly in oils with high initial cis-PUFA content. The higher increase in the accumulation of trans FAs in the present study reflects the extended heating duration, compared to their 10 short frying cycles. Similarly, Zribi *et al.* (2014) reported a decline in cis-MUFAs and cis-PUFAs with a concomitant increase in trans FAs in olive oil and vegetable oil during repeated frying, although the extent of trans FA formation was lower in olive oil because of its higher cis-MUFA content, compared to the seed oils in our study. These findings underscored that the degree of unsaturation and the type of oil critically influence the rate of fatty acid transformation during thermal processing. This was consistent with our results, where linseed oil exhibited the most pronounced compositional changes, attributable to its high content of cis-PUFAs.

Table 2. Effect of (re)heating on fatty acids' (FA) composition (g/100 g total FA) and trans fatty acid formation in different vegetable oils commonly used for frying.

	Heating (h)	C14:0	C16:0	C18:0	C18:1n-9c	C18:1n-9t	C18:2n-6c	C18:2n-6t	C18:3n-3c	SFAs	Total Cis-UFA	Total Cis-PUFA
Canola oil	0	0.11^{c}	6.64^{d}	2.65^{d}	66.3^{a}	0.10^{d}	12.5^{a}	ND	3.26^{a}	9.29^{e}	82.0^{a}	15.7^{a}
	1	0.12^{c}	6.91^{d}	2.67^{d}	64.0^{b}	0.30^{c}	11.9^{b}	ND	3.00^{b}	9.67^{d}	79.0^{b}	14.9^{b}
	6	0.13^{b}	8.30^{c}	3.44^{c}	60.5^{c}	0.39^{c}	11.2^{c}	0.001^{c}	2.49^{c}	11.9^{c}	74.2^{c}	13.7^{c}
	12	0.41^{a}	11.6^{b}	5.21^{b}	55.6^{d}	2.40^{b}	9.14^{d}	0.110^{b}	2.05^{d}	17.3^{b}	66.8^{d}	11.2^{d}
	24	0.40^{a}	19.3^{a}	7.56^{a}	48.0^{e}	5.42^{a}	8.19^{e}	0.500^{a}	0.92^{e}	27.3^{a}	57.1^{e}	9.11^{e}
Corn oil	0	0.11^{e}	6.51^{d}	2.56^{d}	62.8^{a}	0.43^{c}	21.5^{a}	ND	3.80^{a}	9.08d	88.1^{a}	25.2^{a}
	1	0.12^{d}	7.22^{d}	2.59^{d}	59.9^{b}	0.41^{c}	19.2^{b}	ND	3.37^{b}	9.93^{d}	82.5^{b}	22.57^{b}
	6	0.21^{c}	9.40^{c}	2.81^{c}	56.1^{c}	0.40^{c}	14.6^{c}	0.09^{c}	0.37^{c}	12.41^{c}	71.1^{c}	14.97^{c}
	12	0.60^{b}	16.2^{b}	3.19^{b}	50.9^{d}	3.06^{b}	13.5^{d}	0.32^{b}	0.23^{d}	19.96^{b}	64.1^{d}	13.25^{d}
	24	0.93^{a}	34.6^{a}	3.85^{a}	45.2^{e}	3.21^{a}	8.10^{e}	0.50^{a}	0.12^{e}	39.37^{a}	53.3^{e}	8.10^{e}
Sunflower oil	0	ND	10.3^{d}	2.80^{d}	72.9^{a}	1.11^{c}	10.10^{a}	ND	0.46^{a}	13.8^{c}	83.3^{a}	10.5^{a}
	1	ND	10.4^{d}	2.79^{d}	67.6^{b}	1.07^{c}	8.28^{b}	ND	0.25^{b}	13.2^{c}	76.2^{b}	8.52^{b}
	6	ND	11.0^{c}	2.99^{c}	64.7^{c}	1.04^{c}	7.95^{c}	ND	0.10^{c}	13.5^{c}	72.7^{c}	7.95^{c}
	12	ND	20.7^{b}	3.27^{b}	56.4^{d}	3.81^{b}	2.38^{d}	0.21^{b}	0.09^{c}	24.3^{b}	58.8^{d}	2.38^{d}
	24	ND	28.0^{a}	3.84^{a}	52.5^{e}	4.55^{a}	1.66^{e}	0.38^{a}	0.09^{c}	31.9^{a}	54.2^{e}	1.65^{e}
Linseed oil	0	ND	4.72^{e}	1.76^{c}	16.6^{a}	0.00^{e}	8.42^{a}	ND	53.0^{a}	6.65^{e}	78.1^{a}	62.0^{a}
	1	ND	8.32^{d}	1.78^{c}	16.4^{a}	2.00^{d}	7.76^{b}	0.05^{d}	46.1^{b}	10.1^{d}	70.2^{b}	53.0^{b}
	6	ND	17.1^{c}	1.80^{c}	14.5^{b}	3.00^{c}	5.29^{c}	0.12^{c}	38.7^{c}	18.9^{c}	58.5^{c}	44.1^{c}
	12	ND	25.1^{b}	1.96^{b}	13.9^{c}	4.49^{b}	5.59^{d}	0.29^{b}	26.4^{d}	27.1^{b}	45.9^{d}	31.2^{d}
	24	ND	30.6^{a}	2.10^{a}	12.3^{d}	5.72^{a}	4.16^{e}	0.60^{a}	20.0^{e}	32.7^{a}	36.5^{e}	24.5^{e}
SEM		0.002	0.081	0.019	0.299	0.013	0.059	0.001	0.012	0.100	0.36	0.183
Significance												
Heating duration		***	***	***	***	***	***	***	***	***	***	***
Oil type × heating duration		***	***	***	***	***	***	***	***	***	***	***

Notes: The effect of heating duration on FA composition of oils was analyzed with repeated measure ANOVA using PROC MIXED procedure of the Statistical Analysis System (SAS; SAS Institute, NC).

Mean values in the same column within each oil-type with different superscript letters differ at $p < 0.05$.

SEM: standard error of the mean; $^{***}p < 0.001$; ND: not determined.

C14:0: myristic acid; C16:0: palmitic acid; C18:0: stearic acid; C18:1n-9c: oleic acid; C18:2n-6c: linoleic acid; C18:2n-6t: trans isomer of linoleic acid; C18:3n-3c: linolenic acid; total cis-UFA: total unsaturated fatty acids with cis-configuration (C18:1n-9c + C18:2n-6c + C18:3n-3c); total SFAs: total saturated fatty acid (C14:0 + C16:0 + C18:0); total cis-PUFA: total polyunsaturated fatty acids with cis-configuration (C18:2n-6c + C18:3n-3c).

Table 3. Effect of (re)heating on fatty acids' (FA composition (g 100/g total FA) and trans FA formation in commercially hydrogenated vegetable oils (fats) used for frying.

	Heating (h)	C14:0	C16:0	C18:0	C18:1n-9c	C18:1n-9t	C18:2n-6c	C18:2n-6t	C18:3n-3c	SFA	Total Cis-UFA	Total Cis-PUFA
Shama ghee	0	1.21^{c}	46.7^{d}	3.55^{d}	26.2^{a}	12.0^{c}	2.89^{a}	ND	0.05	50.7^{e}	29.7^{a}	2.91^{a}
	1	1.22^{c}	46.6^{d}	3.92^{d}	26.5^{a}	12.8^{c}	2.32^{a}	0.26^{e}	0.00	51.8^{d}	28.8^{b}	2.31^{b}
	6	1.32^{b}	48.8^{c}	4.21^{c}	26.1^{a}	13.8^{b}	1.46^{b}	0.49^{c}	0.00	54.2^{c}	27.5^{c}	1.45^{c}
	12	1.32^{b}	51.3^{b}	5.03^{b}	25.2^{b}	14.9^{b}	$0.81^{b,c}$	0.55^{b}	0.00	57.4^{b}	25.0^{d}	0.81^{d}
	24	1.41^{a}	55.9^{a}	6.12^{a}	$21.\ 3^{c}$	16.6^{a}	0.49^{c}	0.62^{a}	0.00	63.3^{a}	21.81^{e}	0.48^{e}
Kisan ghee	0	0.72^{c}	30.1^{d}	3.55^{c}	47.1^{a}	0.36^{c}	9.52^{a}	ND	0.72^{a}	34.3^{d}	55.9^{a}	9.72^{a}
	1	1.10^{b}	34.1^{c}	3.69^{b}	43.4^{b}	0.36^{c}	8.15^{b}	0.016^{b}	0.62^{a}	39.2^{c}	51.4^{b}	8.87^{b}
	6	1.21^{b}	40.4^{b}	3.69^{b}	39.1^{c}	0.40^{c}	7.95^{b}	0.023^{b}	0.30^{b}	45.2^{b}	48.7^{b}	8.07^{c}
	12	1.27^{a}	50.9^{a}	4.79^{a}	33.9^{d}	1.57^{b}	3.05^{c}	0.060^{a}	0.32^{b}	57.1^{a}	36.4^{c}	3.37^{d}
	24	1.28^{a}	51.9^{a}	4.72^{a}	33.1^{d}	2.10^{a}	2.71^{c}	0.073^{a}	0.29^{b}	57.9^{a}	36.9^{c}	3.00^{d}
Utility ghee	0	1.41^{b}	47.5^{d}	2.98^{c}	37.6^{a}	1.51^{e}	7.21^{a}	ND	0.26^{a}	52.0^{d}	45.1^{a}	2.91^{a}
	1	1.48^{b}	50.9^{c}	3.98^{b}	34.7^{b}	2.23^{d}	2.77^{b}	0.012^{c}	0.27^{a}	56.4^{c}	37.7^{b}	2.31^{b}
	6	$1.71^{a,b}$	50.3^{c}	$5.14^{a,b}$	30.8^{c}	3.21^{c}	1.95^{b}	0.075^{b}	0.27^{a}	56.8^{c}	33.0^{c}	1.46^{c}
	12	1.78^{a}	59.4^{b}	5.95^{a}	22.3^{d}	4.13^{b}	$1.62^{b,c}$	0.093^{a}	0.21^{b}	66.9^{b}	24.2^{d}	0.81^{d}
	24	1.79^{a}	62.1^{a}	6.20^{a}	20.2^{e}	5.65^{a}	1.51^{c}	0.095^{a}	0.21^{b}	69.6^{a}	21.9^{e}	0.49^{d}
Dalda ghee	0	1.33^{c}	46.44^{e}	3.28^{c}	36.1^{a}	0.18^{d}	10.5^{a}	ND	0.34^{a}	51.2^{d}	46.9^{a}	10.87^{a}
	1	1.39^{c}	47.95^{d}	3.29^{c}	36.2^{a}	0.19^{d}	7.61^{b}	ND	0.05^{b}	52.9^{d}	43.9^{b}	7.67^{b}
	6	1.42^{b}	48.73^{c}	4.23^{b}	36.2^{a}	0.58^{c}	5.70^{c}	0.011^{c}	0.04^{b}	54.5^{c}	41.9^{c}	5.70^{c}
	12	1.45^{b}	54.97^{b}	$4.62^{a,b}$	32.1^{b}	2.18^{b}	1.89^{d}	0.116^{b}	0.00^{c}	60.7^{b}	34.0^{d}	1.89^{d}
	24	1.69^{a}	56.88^{a}	4.99^{a}	27.8^{c}	4.03^{a}	0.56^{e}	0.210^{a}	0.00^{c}	63.3^{a}	28.4^{e}	0.55^{e}
SEM		0.034	0.621	0.147	0.402	0.204	0.431	0.001	0.001	0.685	0.749	0.199
Significance												
Heating duration		***	***	***	***	***	***	***	***	***	***	***
Fat type × heating duration		***	***	***	***	***	***	***	***	***	***	***

Notes: The effect of heating duration on the composition of FAs of ghee samples were analyzed with repeated measure ANOVA using PROC MIXED procedure of the statistical analysis system (SAS; SAS Institute, NC, USA).

Mean values in the same column within each oil-type with different superscript letters differ at $p < 0.05$; SEM: standard error of mean; $^{***}p < 0.001$.

C14:0: myristic acid; C16:0: palmitic acid; C18:0: stearic acid; C18:1n-9c: oleic acid; C18:2n-6c: linoleic acid; C18:2n-6t: trans-isomer of linoleic acid; C18:3n-3c: linolenic acid; total cis-UFA: total unsaturated fatty acids with cis-configuration (C18:1n-9c + C18:2n-6c + C18:3n-3c); total SFA: total saturated fatty acid (C14:0 + C16:0 + C18:0); total cis-PUFA: total polyunsaturated fatty acids with cis-configuration (C18:2n-6c + C18:3n-3c).

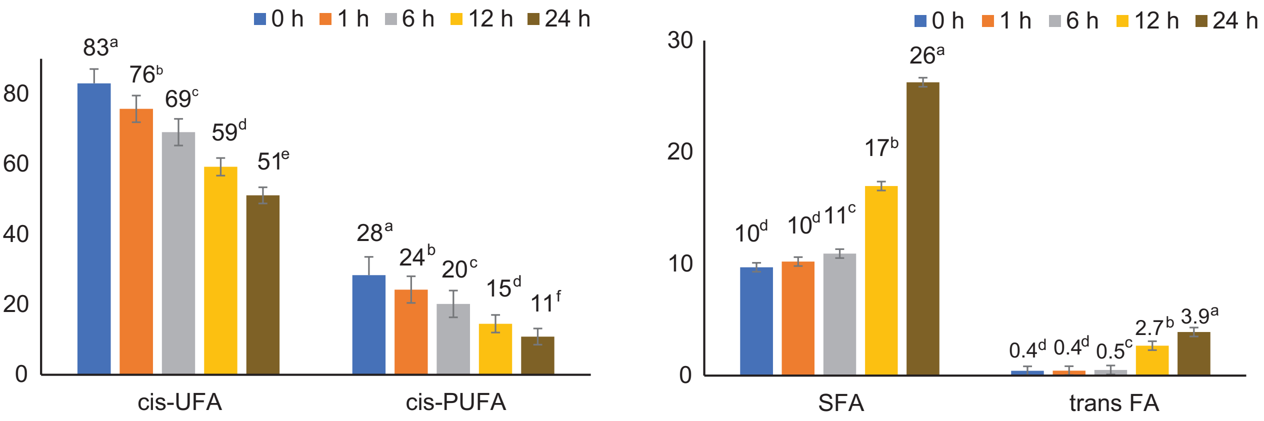

Figure 1. The overall decrease in unsaturated fatty acids (UFA) and polyunsaturated fatty acids (PUFA) contents (Panel A) and increase in saturated fatty acids (SFA) and trans fatty acids (tFA) content (Panel B) during 0, 1, 6, 12 and 24 h heating of the four types of oils.

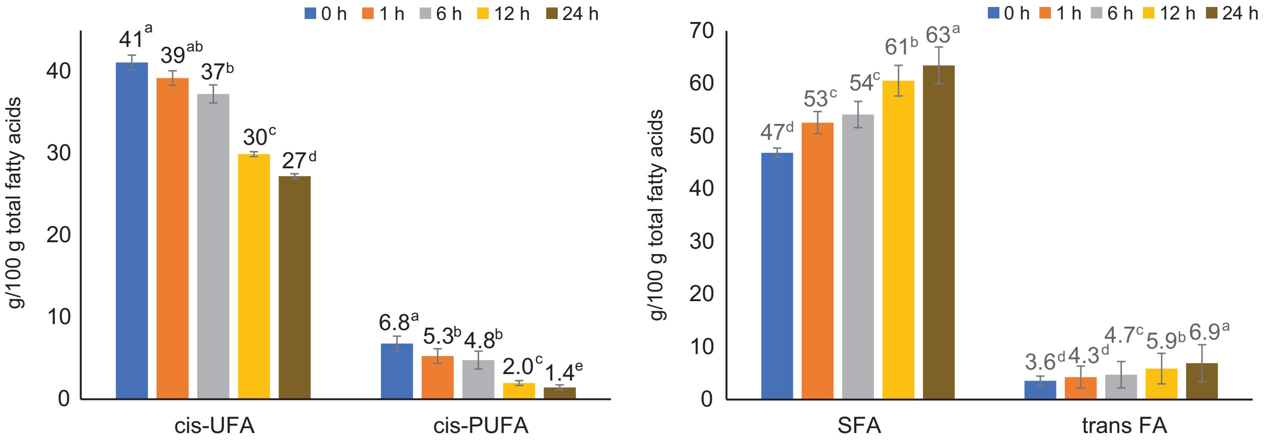

Figure 2. (Panel A) The overall decrease in the contents of total cis-unsaturated fatty acids (cis-UFA) and cis-polyunsaturated fatty acids (cis-PUFA), and (Panel B) increase in the contents of saturated fatty acids (SFA) and trans fatty acids (tFA) during 0-, 1-, 6-, 12- and 24-h heating of four types of hydrogenated fats/ghee. Mean values with different letters differ at $p < 0.05$.

Fats are formed by the partial hydrogenation of edible oils, a process where UFAs are partially converted into SFAs. Industrial hydrogenation also involves heating of edible oils in the presence of catalyst, which converts some cis-double-bonds into trans double bonds, resulting in the formation of trans FAs in fats (Pattanayak, 2019). A large variation was observed in the composition of FAs of unheated fats, probably because of differences in the initial FAs composition of the oils used for hydrogenation, processing temperature, and the extent of hydrogenation.

All unheated oils contained high contents of cis-UFAs (78.6–88.1 g/100 g) and lower contents of SFAs (6.65–13.8 g/100 g) and trans FAs (0–1.1 g/100 g), making their consumption less risky for lifestyle diseases. During extended frying, however, the cis-UFAs in oils were extensively converted into trans FAs because of thermal isomerization and into SFAs because of degradation and polymerization reactions, making the consumption of repeatedly heated oils more risky for lifestyle diseases, such as type-2 diabetes, coronary heart diseases, and sudden cardiac death (Papantoniou *et al.*, 2010). Heating at frying temperatures stimulate isomerization and polymerization reactions that not only destroy double bonds but also induce trans-isomerization in naturally present cis-UFAs (Pattanayak, 2019). In agreement with our findings, Bhardwaj *et al.* (2016) also observed significant increase in SFAs and trans FAs with concomitant decrease in cis-UFAs during repeated heating of oils and fats at 180°C for 30 min and at 220°C for 60 min.

Changes in the composition of FAs of oils and fats, in particular decrease in cis-UFAs (C18:1n-9c, C18:2n-6c, and C18:3n-3c) and increase in SFAs (C16:0 and C18:0) during frying is considered as a good indicator for the level of deterioration in their nutritional quality (Talpur *et al.,* 2012). A relatively smaller SFAs–cis-UFAs ratio is considered good in the long term for human health. For example, unheated oils in the present study had lower SFAs–cis-UFAs ratios (0.09:0.17), compared to unheated fats (0.61:1.71), indicating their higher nutritional quality. Moreover, SFAs–cis-UFAs ratios of all oils and fats increased during 24-h heating at frying temperature, reflecting deterioration in their nutritional quality. We further compared the frying-induced changes in SFAs–cis-UFAs ratios across the oils and fats to highlight variation among individual oils and fats. In the oils, the highest increase in SFAs–cis-UFAs ratio of 0.81 (from 0.09 to 0.90) was recorded in linseed oil, followed by 0.64 (from 0.10 to 0.74) in corn oil; 0.42 (from 0.17 to 0.59) in sunflower oil; and 0.37 (from 0.11 to 0.48) in canola oil. While among fats, the highest increase in SFAs–cis-UFAs ratio was recorded in Utility ghee (2.03; from 1.15 to 3.18), followed by Shama ghee (1.20; from 1.71 to 2.90); Dalda ghee (1.14; from 1.09 to 2.23); and Kisan ghee (0.96; from 0.61 to 1.57). The highest degree of thermal oxidation in linseed oil could be related to its high content of C18:3n-3 and total PUFAs, compared to other analyzed oils. It is reported that thermal oxidation and isomerization of fats and oils is highly related to the degree of unsaturation of their FAs, as the affinity for oxidation increases with increasing unsaturation of FAs (Khan *et al.,* 2009, 2011). Moreover, amount and type of SFAs also affect thermal oxidative stability of oils and fats (Walter and Mozaffarian, 2007).

A consistent decrease in the overall cis-UFAs and cis-PUFAs contents and a consistent increase in SFAs and trans FAs contents across four selected oils and fats with increasing duration of heating highlight consistent deterioration in quality of oils and fats. SFAs, particularly C16:0 and tans FAs, can drive up total cholesterol and increase harmful low-density lipoprotein, thereby enhancing the risks of lifestyle diseases. Moreover, trans FAs easily assimilate and integrate into tissue lipids, as human lipase enzyme works only on cis-configuration and cannot metabolize trans FAs (Brundiek *et al.,* 2012). The risk of heart disease increases by 23% for every 2% increase in the daily calories of trans fat; therefore, even a very tiny amount of consumption of trans fat could be detrimental to human health. These findings demonstrate that repeated heating of oils and fats must be avoided. This information could also be used for developing basic regulations for maintaining quality of oils and fats during frying of foods.

Conclusions

This current study reports the first inclusive dataset on changes in the composition of FAs and accumulation of trans FAs in fats and oils subjected to repeated frying for different durations, thereby reflecting the deep-frying practices adopted by homemakers and commercial vendors in Pakistan. Despite large variability in FAs contents, repeated frying for 24 h consistently decreased the contents of cis-UFAs and increased the contents of SFAs and trans FAs in all fats and oils. Moreover, the SFAs–cis-UFA ratio of all fats and oils increased during 24-h heating, reflecting deterioration in their nutritional quality, demonstrating that extended frying must be avoided to prevent undesirable changes in FA composition. Local guidelines must be developed to stop the practice of reusing the same fat/oil and maintaining a specific temperature during frying by homemakers and commercial vendors.

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Author Contributions

Conceptualization, Sahib Alam; methodology, Nazir Ahmad Khan; software, Muhammad Suleman; validation, Nawal Al-Hoshani and Fakhria A. Al-Joufi; formal analysis, Asma Khan; investigation, Muhammad Nauman Ahmad; resources, Tariq Aziz; data curation, Ayaz Ali Khan and Taqweem ul Haq.; writing—original draft preparation, Nazir Ahmad Khan and Asma Khan; writing—review and editing, Afia Zia; visualization, Fahad Al-Asmari; supervision, Tariq Aziz and Sahib Alam.; project administration, Sahib Alam; funding acquisition, Tariq Aziz.

Conflict of Interest

The authors declared no conflict of interest.

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