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Temperature-driven alterations in *trans* octadecanoic acid isomers of high-TFA soybean oil during heating

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Abstract

Trans-fatty acid (TFA) formation and degradation during heating in real-life situations remain a major public-health concern, particularly in countries where soybean oil contains elevated baseline TFA levels. This study examined temperature-driven alterations in C18 *cis*/*trans* isomers in soybean oil rich in TFA (3.35%) during continuous heating at 180±5°C for 8 h. Fatty-acid profiles were quantified hourly using GC-FID with *cis*/*trans* standard calibration. Continuous heating steadily changed polyunsaturated fatty acids (PUFA) to more saturated (SFA) and monounsaturated (MUFA) fractions, driven by PUFA degradation ($p < 0.001$). Total TFA (TTFA) content showed no significant net change ($p=0.152$), masking isomer-specific dynamics. *Trans*-oleic acid (TOA) increased steadily ($R^2 = 0.988$; $p < 0.001$), indicating *cis* to *trans* isomerization; *trans*-linoleic acid (TLA) rose modestly due to increases in *trans*, *trans* and *cis*, *trans* isomers; while *trans*-linolenic acid (TLnA) declined sharply ($p < 0.01$), consistent with preferential oxidation of highly unsaturated *trans* isomers. Multivariate analyses supported these dynamics. Hierarchical clustering separated fatty acids into: (i) SFA-MUFA-TOA (increasing, thermally stable), (ii) PUFA-TLnA (highly degradable), and (iii) TLA-TTFA (non-linear behavior). Principal Component Analysis (PC1 = 82.1%, PC2 = 15.1%) showed a clear time-dependent trajectory driven by PUFA loss and accumulation of SFA/MUFA and selected *trans* isomers.

Keywords: Soybean oil; *Trans* fatty acids; *Trans* linolenic acids; *Trans* linoleic acids; *Cis*-*trans* isomerization

Introduction

Recently *trans* fats are able to catch the attention of food researchers, experts and physicians after being documented as a potential health hazard for humans (Mozaffarian *et al.* 2006). *Trans* fats or *trans* fatty acids (TFA) refers to any unsaturated fatty acid containing at least one hydrogen at *trans* configuration in their carbon chain. Due to this *trans* isomeric configuration TFAs have higher melting point and greater thermodynamic stability than its *cis* form. These *trans* isomers of fatty acids are more stable than their *cis* counterparts and results from reactions when fats are heated at high temperature for a long time (Moya Moreno *et al.* 1999). Researchers found that TFAs raise low-density lipoprotein (LDL) and reduces high-density lipoprotein (HDL), which leads to increased risk of coronary heart diseases (CHD) (Bhat *et al.* 2022). It was found that a 2% rise in *trans*-fat intake has been linked with a 23% higher risk of developing CHD (Mozaffarian and Clarke, 2009). Additionally, consuming 5 g of *trans* fats per day is related with a 25% increase in the likelihood of ischemic heart disease (Marklund *et al.* 2025; Mozaffarian *et al.* 2006; Stender *et al.* 2006). Hence, national and international agencies setup guideline to limit TFAs in edible oil not more than 2% and recommended calories from TFAs should not be higher than 1% (Bangladesh Food Safety Authority (BFSA), 2021) (World Health Organization (WHO), 2018).

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Heating oil at high temperature (above 160°C) results in long chain unsaturated fatty acids undergo hydroperoxide breakdown by β-scission and produce low molecular weight volatile compounds, non-volatile compounds like aldehydes, ketones and short chain fatty acids (Berdeaux *et al.* 2012; Márquez-Ruiz and Dobarganes, 2007). Various studies described that heating above 180°C slightly increases the concentration of TFAs in different vegetable oils (Li *et al.* 2012). On the other hand, some studies found heating at or below 180°C does not affect TFA content (Liu *et al.* 2007). However, most of the time control oil does not contain a significant amount of TFA prior to heating. In Bangladesh, vegetable oils, particularly soybean oil, undergo a high temperature heat treatment throughout the deodorization step. In the deodorization step, the oil sample is heated above 240°C for 3-4 hours, which increases the TFA content up to 4.8% of the oil (Bhardwaj *et al.* 2016; Sarwar *et al.* 2024; Tasan and Demirci, 2003).

Soybean oil is the most popularly consumed household cooking oil in Bangladesh, where it is routinely heated for sautéing, deep-frying, and prolonged cooking. Recent national assessments have revealed that commercially refined soybean oils often contain elevated levels of TFAs particularly *trans*-linoleic and *trans*-linolenic acid isomers-formed during high-temperature refining and deodorization (Kabir *et al.* 2025; Sarwar *et al.* 2024). Although several controlled laboratory studies have documented the formation of TFAs during thermal treatment of vegetable oils, no published research has examined how additional household-level heating affects the stability or further transformation of *trans*-octadecanoic acid isomers in already high-TFA containing soybean oils. This gap is especially important for Bangladesh, where consumers unknowingly purchase oils with substantial pre-existing TFA content and then expose them to extended cooking temperatures that may intensify or modify *trans*-isomer profiles.

Therefore, the present study aims to investigate the temperature-driven changes in *trans*-C18 isomers in high-TFA soybean oil under heating conditions that closely mimic real household practices. This work provides essential evidence for public-health risk assessment, supports national regulatory actions, and contributes mechanistic insight into the thermal behavior of pre-formed *trans* isomers in edible oils.

Materials and methods

Materials

Commercially available soybean oil from three brands were collected from the local market and transferred to the laboratory. A composite soybean oil was produced by mixing these three oil samples and stored in three oil containers of two liters. The oil containers were stored in dark until the experiment was performed. A mixed standard of octadecanoic acid *cis*/*trans* isomers were prepared using methyl oleate (C 18:1 c9), methyl elaidate (C 18:1 t9), linoleic acid methyl ester mix (C 18:2 isomers) and Linolenic acid methyl ester mix (C 18:3 isomers) (Supelco, Bellefonte, PA, USA). A Supelco 37 Component FAME Mix (Supelco, Bellefonte, PA, USA) was used to identify other fatty acids. All other chemicals and solvents of analytical grade were purchased from Merck (Darmstadt, Germany).

Heating procedure

Two liters of composite soybean oil samples were poured in a traditional aluminum frying pan and placed on a gas burner. The gas burner was set at a point that the temperature of oil remained 180±5°C. The heating time counted after the oil temperature adjusted at 180±5°C and heating continued for eight hours with occasional stirring to ensure even temperature rise. Before heating started, a 50 mL oil sample was collected in a 50 mL sample bottle which was considered a control sample. During heating, 50 mL of oil sample was collected in a 50 mL sample bottle every hour, cooled to room temperature, and kept in the refrigerator for further analysis.

Preparation of fatty acid methyl ester (FAME)

Fatty acids in oils were converted into corresponding FAMEs by following ISO 12966-2:2017(E) with slight modification as described by Lisa *et al.* (2024). In brief, about 20 mg of oil sample was transferred into a test tube. A volume of 2 mL isooctane was added to the oil aliquot and shaken vigorously till total solubilization. With the solution, 0.2 ml of 2 M methanolic KOH was added and properly shaken for 1 minute; then 2 mL aqueous saturated sodium chloride was added and centrifuged for 30 seconds at 600g for phase separation. The clear upper portion was then passed through anhydrous sodium sulphate column and collected in a 1.5 ml glass vial for GC analysis.

Gas chromatography

Identification and quantification of FAMEs of the oils were conducted using Thermo Fisher Scientific Gas Chromatograph (Trace 1300, MA, USA) coupled with flame ionization

detector (FID), thermo AI 1310 autosampler and a highly polar fused silica capillary column (Rt-2560, Restek, Bellefonte, PA, USA) (100 m × 0.25 mm i.d × 0.20 µm film thickness) was used for fatty acid methyl ester separation. In this experiment, prepared FAME samples were injected in a 20:1 split mode using ultrapure nitrogen as carrier gas at a constant flow of 1mL/min. The injector temperature was maintained at 250°C. The GC oven temperature program began at 120°C, held for 10 minutes, followed by an increase rate of 16°C/min to 200°C and hold for 15 minutes. Then, the temperature was again increased to 240°C at 16°C/min and kept isothermal for 10 minutes. The results generated by an automatic software, prepared for GC analysis (Chromeleon, version 7.00), were reported as relative peak percentage by matching fatty acid fractions against reference standards including Supelco 37 component FAME mix, C 18:2 isomer mix and 18:3 isomer mix (Supelco, Bellefonte, PA, USA). Fatty acid identification and quantification were performed by comparing the retention times and peak areas with those of the respected FAME standards. During the experiment, it was found that two fatty acids namely eicosanoic acid (20:0) and eicosenoic acid (20:1) were coeluted with two *trans* standard all *trans* linolenic acid (18:3 9t12t15t) and one isomer of *trans* linolenic acid (18:3 9t,12c,15c) respectively. To analyze *trans*-fat in oil precisely, a column of different polarity (FAMEWAX, Restek, Bellefonte, PA, USA) (30 m × 0.25 mm i.d × 0.25 µm film thickness) was used in this experiment and the concentration of eicosanoic acid (20:0) and eicosenoic acid (20:1) was measured accurately. By deducting the amount of these two fatty acids from the total TFA, the actual amount of TFA was measured.

Statistical analysis

Heating experiment was conducted three times and TFAs were analyzed in a single run for all oil samples. Data are presented as mean ± SD. Data were analyzed by ANOVA, Duncan's test, linear regression, HCA, and PCA using Origin software.

Results and discussion

Identification and quantification of eicosanoic acid (C 20:0) and eicosenoic acid (C 20:1)

From Fig. 2. (a) and (b), it was found that these two fatty acids were coeluted with two *trans* linolenic acid isomers. Hence, it is crucial to quantify the amount of C 20:0 and C 20:1 in oil samples. These two compounds were identified and quantified using another column of different polarity (Fig. 2. c) and it was found that refined soybean oil contained 0.39 g/100 g and 0.21 g/100g of C 20:0 and C 20:1 fatty acid respectively. It was also found that these two fatty acids remained unchanged during the heating experiment. This statement is supported by Li *et al.* 2012 who also found a similar amount of C 20:0 and C 20:1 in soybean oil.

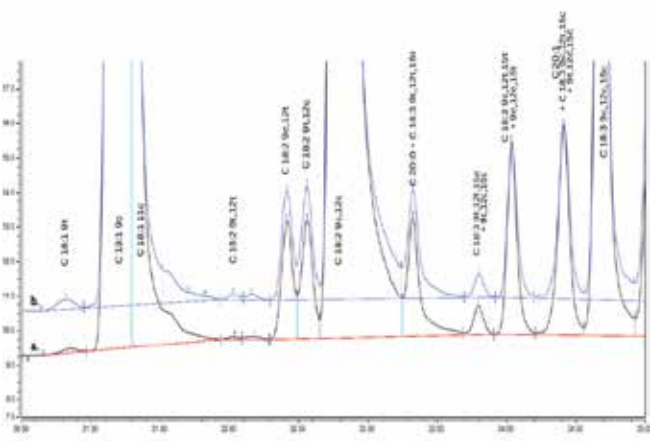

Fig. 1. Partial chromatogram of soybean oil a) Control b) Heated for 8 hours

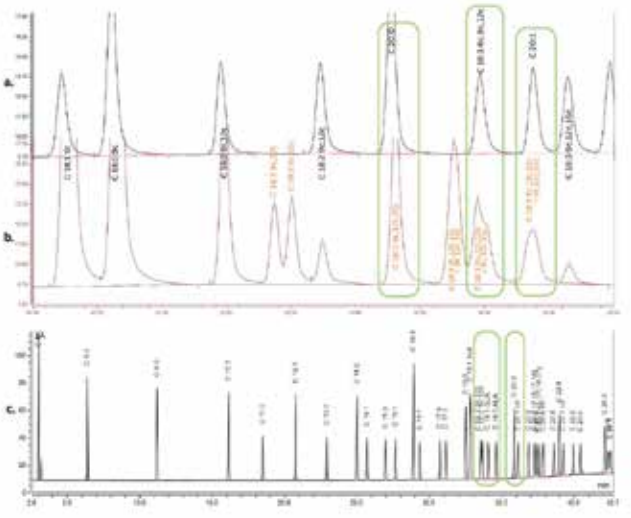

Fig. 2. Chromatogram of a) Supelco 37 Component FAME mix in Rt-2560 column; b) Octadecanoic acid cis/trans isomers methyl ester mix in Rt-2560 column and c) Supelco 37 Component FAME mix in FAMEWAX column

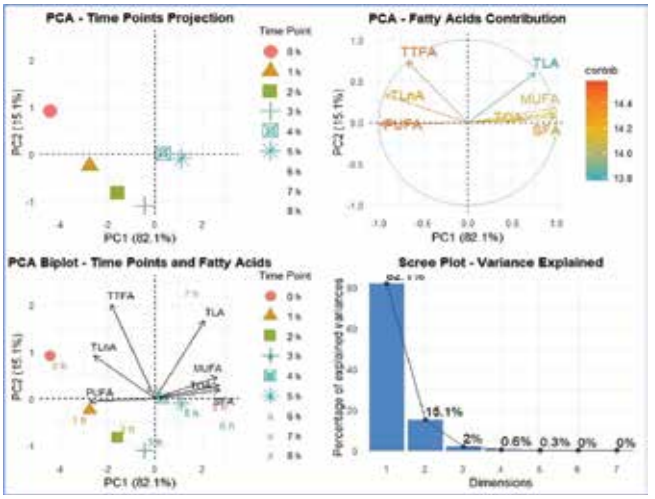

Fig. 3. Principal component analysis (PCA) of fatty acid composition over time. (A) Scores plot showing temporal trajectory from 0h to 8h, (B) Loading plot displaying fatty acid contributions, (C) Biplot combining samples and variables, and (D) Scree plot indicating variance explained by principal components

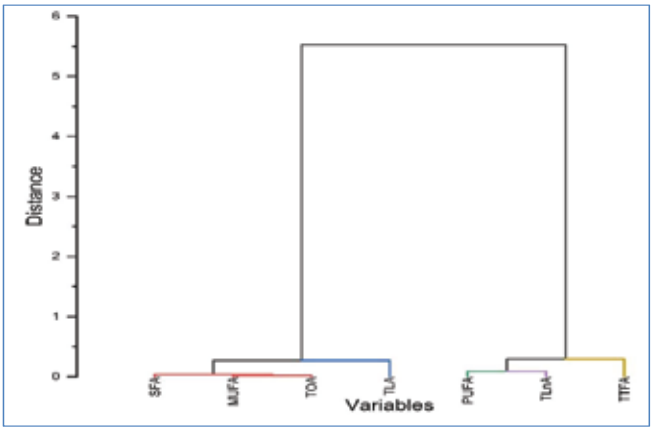

Fig. 4. Hierarchical clustering of grouped fatty acid variables The hierarchical cluster analysis (HCA) grouped the fatty acids into three distinct clusters based on their response to thermal stress: (1) SFA, MUFA, and TOA formed a cluster characterized by a consistent increase, indicating relative stability or accumulation; (2) PUFA and TLnA clustered together due to their pronounced decrease, highlighting their high susceptibility to degradation; and (3) TLA and TTFA formed a separate cluster with fluctuating patterns, suggesting complex, non-linear kinetics for trans isomer formation. This clustering validates the systematic reorganization of the oil's fatty acid profile during repeated heating

Overall changes in fatty acid pattern

Heating soybean oil at 180±5°C for 8 h caused substantial and highly systematic modifications in its fatty acid profile. The unheated control oil exhibited a PUFA-rich composition (PUFA = 61.11%), dominated by linoleic acid (LA, 52.60%) and α-linolenic acid (LnA, 4.99%). However, both PUFAs showed pronounced thermal degradation, decreasing to 50.05% and 4.21%, respectively, after 8 h (Table I). Regression analysis confirmed strong linear decline for LA (slope = –0.304%/h; R^2 = 0.984; $p < 0.001$) and LnA (slope = –0.090%/h; R^2 = 0.900; $p < 0.001$). These observations are consistent with established reports showing that the bis-allylic protons in LA and LnA render them the first targets for hydrogen abstraction and β-scission during thermal oxidation (Choe and Min, 2007; Nayak *et al.* 2015). On the other hand, steadily increase in saturated fatty acids (SFA) from 15.65% to 17.85% (R^2 = 0.950), which was due to the increment of palmitic acid (C 16:0), from 10.90% to 11.92% (slope = +0.129%/h) and stearic acid, increased from (3.59% to 4.58%; p = 0.002), consistent with previous thermal-oxidation observations in soybean oil (Shen *et al.* 2021).

Monounsaturated fatty acids (MUFA) showed a smaller yet significant increase (23.24% to 24.51%; slope = +0.162%/h; R^2 = 0.987), driven largely by oleic acid (C18:1 c9). MUFAs are thermally more stable than PUFAs and can accumulate proportionally as PUFA degrade, matching earlier reports by Kabir *et al.* 2025. This increase is not due to de novo formation but reflects relative enrichment, as PUFA degradation lowers the proportional contribution of stable SFA (Abbas Ali *et al.* 2017; Liu *et al.* 2024).

The systematic decrease in LA and LnA reflects successive oxidative steps-hydrogen abstraction, formation of peroxyl radicals, and β-scission-which cleaves double-bond adjacent C-C bonds, generating shorter fragments such as C8:0, C9:0 aldehydes, or volatile hydrocarbons. Supporting this mechanism, caprylic acid (C8:0) increased from non-detectable levels to 0.15% after 8 h (slope = +0.020%/h; R^2 = 0.995) (Supplementary Table II). This mirrors the classical β-scission pathways described by (Bazina *et al.* 2025; Frankel, 2005), where linoleate hydroperoxides decompose into octanoate and hexanal.

Formation and transformation of TFAs during heating

Despite the presence of high TFA in the starting oil (3.35%), likely due to high-temperature deodorization as documented by Kabir *et al.* 2025 TTFA showed no significant linear overall change (p = 0.152). However, isomer-specific changes reveal complex dynamics: *trans* oleic acid (TOA) increased steadily (0.03% → 0.08%; R^2 = 0.988; $p < 0.001$), consistent with *cis* to *trans*

isomerization at high temperature. *Trans* linoleic acid (TLA) increased slightly (1.17% to 1.19%; slope = +0.005%/h), mainly driven by TLA-tt and TLA-ct, whereas TLA-tc remained unchanged. *Trans* linolenic isomers (TLnA) decreased significantly across all sub-isomers ($p < 0.01$), indicating preferential oxidation of TLnA compared to TLA (Fig 1). The cumulative outcome is a flat TTFA value that conceals considerable internal reconfiguration of the individual *trans* isomers, as has also been found by Song *et al.* (2015).

Table I. Percent changes in octadecanoic acids during heating

Fatty Acids	Heating hour								
	0 h	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
C 18:0	3.59 (±0.11)	3.90 (±0.09)	4.31 (±0.12)	4.36 (±0.15)	4.42 (±0.21)	4.45 (±0.12)	4.51 (±0.18)	4.54 (±0.84)	4.58 (±0.15)
TOA	0.03 (±0.01)	0.04 (±0.01)	0.04 (±0.01)	0.05 (±0.01)	0.06 (±0.01)	0.06 (±0.01)	0.07 (±0.01)	0.07 (±0.02)	0.08 (±0.02)
C 18:1 c9	20.66 (±0.55)	20.79 (±0.62)	20.85 (±0.35)	21.11 (±0.67)	21.35 (±0.38)	21.43 (±0.42)	21.56 (±0.54)	21.67 (±2.54)	21.84 (±0.67)
C 18:1 11c	2.20 (±0.12)	2.22 (±0.15)	2.30 (±0.14)	2.29 (±0.21)	2.05 (±0.17)	2.27 (±0.20)	2.24 (±0.19)	2.28 (±0.54)	2.30 (±0.24)
TLA tt	0.01 (±0.01)	0.01 (±0.01)	0.02 (±0.01)	0.02 (±0.01)	0.02 (±0.01)	0.03 (±0.01)	0.03 (±0.01)	0.03 (±0.01)	0.03 (±0.01)
TLA tc	0.57 (±0.03)	0.56 (±0.02)	0.56 (±0.07)	0.56 (±0.04)	0.57 (±0.05)	0.57 (±0.11)	0.57 (±0.15)	0.57 (±0.21)	0.56 (±0.13)
TLA ct	0.59 (±0.04)	0.59 (±0.04)	0.59 (±0.05)	0.59 (±0.03)	0.60 (±0.08)	0.60 (±0.05)	0.60 (±0.07)	0.63 (±0.19)	0.60 (±0.11)
LA cc	52.60 (±1.01)	52.18 (±1.18)	51.68 (±1.05)	51.38 (±1.32)	51.26 (±1.52)	50.81 (±1.09)	50.66 (±1.21)	50.36 (±4.22)	50.05 (±1.28)
TLnA ttt	0.31 (±0.01)	0.29 (±0.03)	0.29 (±0.05)	0.30 (±0.09)	0.31 (±0.07)	0.32 (±0.05)	0.31 (±0.05)	0.32 (±0.12)	0.33 (±0.09)
TLnA ttc+tct	0.13 (±0.01)	0.13 (±0.02)	0.12 (±0.01)	0.12 (±0.02)	0.12 (±0.02)	0.12 (±0.02)	0.11 (±0.03)	0.12 (±0.04)	0.12 (±0.01)
TLnA ctt+cct	0.83 (±0.08)	0.82 (±0.05)	0.80 (±0.05)	0.78 (±0.06)	0.77 (±0.07)	0.76 (±0.08)	0.74 (±0.07)	0.77 (±0.24)	0.73 (±0.12)
TLnA ctc+tcc	0.88 (±0.02)	0.85 (±0.04)	0.83 (±0.07)	0.81 (±0.04)	0.81 (±0.08)	0.79 (±0.07)	0.76 (±0.09)	0.78 (±0.21)	0.78 (±0.11)
LnA ccc	4.99 (±0.26)	4.86 (±0.31)	4.72 (±0.19)	4.58 (±0.33)	4.53 (±0.18)	4.40 (±0.21)	4.28 (±0.35)	4.45 (±0.97)	4.21 (±0.51)
SFA	15.65 (±1.21)	16.11 (±1.05)	16.65 (±0.95)	16.89 (±1.60)	17.13 (±1.09)	17.31 (±0.98)	17.52 (±1.23)	17.71 (±3.03)	17.85 (±0.99)

Continued

MUFA	23.24 (±2.06)	23.34 (±1.81)	23.48 (±1.98)	23.74 (±1.52)	23.74 (±2.01)	24.05 (±1.88)	24.16 (±1.09)	24.32 (±2.81)	24.51 (±1.67)
PUFA	61.11 (±3.02)	60.50 (±2.72)	59.82 (±2.11)	59.33 (±1.79)	59.11 (±2.18)	58.60 (±1.91)	58.27 (±1.69)	58.27 (±4.66)	57.60 (±1.65)
TTFA	3.35 (±0.21)	3.29 (±0.32)	3.26 (±0.37)	3.24 (±0.19)	3.26 (±0.18)	3.25 (±0.33)	3.20 (±0.28)	3.32 (±0.85)	3.22 (±0.29)
TOA	0.03 (±0.01)	0.04 (±0.01)	0.04 (±0.01)	0.05 (±0.02)	0.06 (±0.02)	0.06 (±0.02)	0.07 (±0.03)	0.07 (±0.05)	0.08 (±0.04)
TLA	1.17 (±0.09)	1.17 (±0.11)	1.17 (±0.15)	1.17 (±0.14)	1.19 (±0.21)	1.19 (±0.18)	1.20 (±0.16)	1.22 (±0.32)	1.19 (±0.09)
TLnA	2.15 (±0.22)	2.08 (±0.19)	2.04 (±0.21)	2.01 (±0.18)	2.01 (±0.15)	1.99 (±0.16)	1.93 (±0.25)	2.02 (±0.32)	1.96 (±0.18)

Table II. Changes in fatty acid profile of high-trans soybean oil during 8 h heating

Fatty Acids	Heating hour								
	0 h	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
C 8:0	0.00	0.02 (±0.01)	0.04 (±0.01)	0.06 (±0.01)	0.09 (±0.02)	0.10 (±0.03)	0.12 (±0.03)	0.14 (±0.04)	0.15 (±0.05)
C 16:0	10.90 (±0.52)	11.03 (±0.23)	11.14 (±0.91)	11.30 (±0.61)	11.47 (±0.12)	11.57 (±0.77)	11.69 (±0.51)	11.80 (±1.22)	11.92 (±0.51)
C 18:0	3.59 (±0.11)	3.90 (±0.09)	4.31 (±0.12)	4.36 (±0.15)	4.42 (±0.21)	4.45 (±0.12)	4.51 (±0.18)	4.54 (±0.84)	4.58 (±0.15)
TOA	0.03 (±0.01)	0.04 (±0.01)	0.04 (±0.01)	0.05 (±0.01)	0.06 (±0.01)	0.06 (±0.01)	0.07 (±0.01)	0.07 (±0.02)	0.08 (±0.02)
C 18:1 c9	20.66 (±0.55)	20.79 (±0.62)	20.85 (±0.35)	21.11 (±0.67)	21.35 (±0.38)	21.43 (±0.42)	21.56 (±0.54)	21.67 (±2.54)	21.84 (±0.67)
C 18:1 11c	2.20 (±0.12)	2.22 (±0.15)	2.30 (±0.14)	2.29 (±0.21)	2.05 (±0.17)	2.27 (±0.20)	2.24 (±0.19)	2.28 (±0.54)	2.30 (±0.24)
TLA tt	0.01 (±0.01)	0.01 (±0.01)	0.02 (±0.01)	0.02 (±0.01)	0.02 (±0.01)	0.03 (±0.01)	0.03 (±0.01)	0.03 (±0.01)	0.03 (±0.01)
TLA tc	0.57 (±0.03)	0.56 (±0.02)	0.56 (±0.07)	0.56 (±0.04)	0.57 (±0.05)	0.57 (±0.11)	0.57 (±0.15)	0.57 (±0.21)	0.56 (±0.13)
TLA ct	0.59 (±0.04)	0.59 (±0.04)	0.59 (±0.05)	0.59 (±0.03)	0.60 (±0.08)	0.60 (±0.05)	0.60 (±0.07)	0.63 (±0.19)	0.60 (±0.11)
LA cc	52.60 (±1.01)	52.18 (±1.18)	51.68 (±1.05)	51.38 (±1.32)	51.26 (±1.52)	50.81 (±1.09)	50.66 (±1.21)	50.36 (±4.22)	50.05 (±1.28)
C 20:0	0.39 (±0.04)	0.39 (±0.03)	0.40 (±0.03)	0.40 (±0.04)	0.40 (±0.04)	0.40 (±0.05)	0.41 (±0.08)	0.41 (±0.22)	0.41 (±0.05)
	0.31	0.29	0.29	0.30	0.31	0.32	0.31	0.32	0.33

Continued

TLnA ttt	(±0.01)	(±0.03)	(±0.05)	(±0.09)	(±0.07)	(±0.05)	(±0.05)	(±0.12)	(±0.09)
	0.13	0.13	0.12	0.12	0.12	0.12	0.11	0.12	0.12
TLnA ttc+tct	(±0.01)	(±0.02)	(±0.01)	(±0.02)	(±0.02)	(±0.02)	(±0.03)	(±0.04)	(±0.01)
TLnA	0.83	0.82	0.80	0.78	0.77	0.76	0.74	0.77	0.73
ctt+cct	(±0.08)	(±0.05)	(±0.05)	(±0.06)	(±0.07)	(±0.08)	(±0.07)	(±0.24)	(±0.12)
	0.21	0.21	0.21	0.21	0.22	0.21	0.22	0.23	0.22
C 20:1	(±0.01)	(±0.01)	(±0.02)	(±0.02)	(±0.02)	(±0.04)	(±0.02)	(±0.05)	(±0.03)
TLnA	0.88	0.85	0.83	0.81	0.81	0.79	0.76	0.78	0.78
ctc+tcc	(±0.02)	(±0.04)	(±0.07)	(±0.04)	(±0.08)	(±0.07)	(±0.09)	(±0.21)	(±0.11)
	4.99	4.86	4.72	4.58	4.53	4.40	4.28	4.45	4.21
LnA ccc	(±0.26)	(±0.31)	(±0.19)	(±0.33)	(±0.18)	(±0.21)	(±0.35)	(±0.97)	(±0.51)
	0.05	0.05	0.04	0.04	0.02	0.04	0.05	0.04	0.04
C 20:2	(±0.01)	(±0.01)	(±0.01)	(±0.01)	(±0.02)	(±0.02)	(±0.01)	(±0.02)	(±0.01)
	0.40	0.40	0.40	0.41	0.42	0.42	0.43	0.45	0.43
C 22:0	(±0.05)	(±0.07)	(±0.08)	(±0.04)	(±0.09)	(±0.10)	(±0.09)	(±0.15)	(±0.09)
	0.11	0.11	0.12	0.10	0.08	0.11	0.10	0.12	0.12
C 22:2	(±0.01)	(±0.01)	(±0.01)	(±0.01)	(±0.02)	(±0.02)	(±0.05)	(±0.05)	(±0.01)
	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.16
C 24:0	(±0.03)	(±0.02)	(±0.01)	(±0.01)	(±0.03)	(±0.03)	(±0.04)	(±0.04)	(±0.02)
	15.65	16.11	16.65	16.89	17.13	17.31	17.52	17.71	17.85
SFA	(±1.21)	(±1.05)	(±0.95)	(±1.60)	(±1.09)	(±0.98)	(±1.23)	(±3.03)	(±0.99)
	23.24	23.34	23.48	23.74	23.74	24.05	24.16	24.32	24.51
MUFA	(±2.06)	(±1.81)	(±1.98)	(±1.52)	(±2.01)	(±1.88)	(±1.09)	(±2.81)	(±1.67)
	61.11	60.50	59.82	59.33	59.11	58.60	58.27	58.27	57.60
PUFA	(±3.02)	(±2.72)	(±2.11)	(±1.79)	(±2.18)	(±1.91)	(±1.69)	(±4.66)	(±1.65)
	3.35	3.29	3.26	3.24	3.26	3.25	3.20	3.32	3.22
TTFA	(±0.21)	(±0.32)	(±0.37)	(±0.19)	(±0.18)	(±0.33)	(±0.28)	(±0.85)	(±0.29)
	0.03	0.04	0.04	0.05	0.06	0.06	0.07	0.07	0.08
TOA	(±0.01)	(±0.01)	(±0.01)	(±0.02)	(±0.02)	(±0.02)	(±0.03)	(±0.05)	(±0.04)
	1.17	1.17	1.17	1.17	1.19	1.19	1.20	1.22	1.19
TLA	(±0.09)	(±0.11)	(±0.15)	(±0.14)	(±0.21)	(±0.18)	(±0.16)	(±0.32)	(±0.09)
	2.15	2.08	2.04	2.01	2.01	1.99	1.93	2.02	1.96
TLnA	(±0.22)	(±0.19)	(±0.21)	(±0.18)	(±0.15)	(±0.16)	(±0.25)	(±0.32)	(±0.18)

Table III. Linear regression analysis of fatty acid changes during heating

Fatty Acid	Slope (%/hour)	R^2	p-value	Significance	Trend
C 8:0	0.02	0.995	<0.001	***	Increasing
C 14:0	0.001	0.931	<0.001	***	Increasing
C 16:0	0.129	0.996	<0.001	***	Increasing
C 18:0	0.106	0.768	0.002	**	Increasing
C 18:1 11c	0.007	0.055	0.544	ns	Increasing
C 18:1 c9	0.152	0.984	<0.001	***	Increasing
C 20:0	0.006	0.988	<0.001	***	Increasing
C 20:1	0.002	0.858	<0.001	***	Increasing
C 20:2	-0.001	0.035	0.631	ns	Decreasing
C 20:3 n6	-0.001	0.043	0.594	ns	Decreasing
C 22:0	0.005	0.844	<0.001	***	Increasing
C 22:1 n9	-0.001	0.073	0.482	ns	Decreasing
C 22:2	0.001	0.014	0.764	ns	Increasing
C 23:0	-0.002	0.428	0.056	ns	Decreasing
C 24:0	0.002	0.857	<0.001	***	Increasing
LA cc	-0.304	0.984	<0.001	***	Decreasing
LnA ccc	-0.09	0.9	<0.001	***	Decreasing
TLA ct	0.003	0.501	0.033	*	Increasing
TLA tc	0				No change
TLA tt	0.002	0.883	<0.001	***	Increasing
TLnA ctc tcc	-0.013	0.863	<0.001	***	Decreasing
TLnA ctt+cct	-0.012	0.89	<0.001	***	Decreasing
TLnA ttc+tct	-0.002	0.663	0.008	**	Decreasing
TLnA ttt	0.006	0.572	0.018	*	Increasing
TOA	0.006	0.988	<0.001	***	Increasing
MUFA	0.162	0.987	< 0.001	***	Increasing
PUFA	-0.409	0.967	< 0.001	***	Decreasing
SFA	0.263	0.95	< 0.001	***	Increasing
TLA	0.005	0.661	0.008	**	Increasing
TLnA	-0.02	0.689	0.006	**	Decreasing
TOA	0.006	0.972	< 0.001	***	Increasing
TTFA	-0.009	0.27	0.152	ns	Decreasing

Variable	PC1_Loading	PC2_Loading	PC1_Contribution	PC2_Contribution	PC1_Correlation	PC2_Correlation
SFA	0.9878	0.0649	16.99	0.4	0.9878	0.0649
MUFA	0.9636	0.1699	16.16	2.72	0.9636	0.1699
PUFA	-0.9939	-0.0189	17.2	0.03	-0.9939	-0.0189
TTFA	-0.6731	0.7338	7.89	50.83	-0.6731	0.7338
TOA	0.9797	0.1035	16.71	1.01	0.9797	0.1035
TLA	0.7567	0.6041	9.97	34.45	0.7567	0.6041
TLnA	-0.9309	0.3344	15.09	10.55	-0.9309	0.3344

PCA Loading Scores

Multivariate pattern recognition (PCA and HCA)

The hierarchical cluster analysis (HCA) grouped the fatty acids into three distinct clusters based on their response to thermal stress: (1) SFA, MUFA, and TOA formed a cluster characterized by a consistent increase, indicating relative stability or accumulation; (2) PUFA and TLnA clustered together due to their pronounced decrease, highlighting their high susceptibility to degradation; and (3) TLA and TTFA formed a separate cluster with fluctuating patterns, suggesting complex, non-linear kinetics for *trans* isomer formation. This clustering validates the systematic reorganization of the oil's fatty acid profile during repeated heating.

PCA revealed clear temporal dynamics in fatty acid composition, with the first two principal components explaining 97.2% of total variance (PC1: 82.1%, PC2: 15.1%), indicating great systematic changes over time. PC1 primarily represented a transition from high PUFA content (loading: -0.994) to increasing SFA (loading: 0.988) and MUFA (loading: 0.964) levels, exhibiting a clear time-dependent trajectory from 0 h to 8 h. PC2 captured variations in minor fatty acids, particularly TTFA (loading: 0.734). The analysis further demonstrated that TOA (loading: 0.980) and TLA (loading: 0.757) showed a strong increase with PC1, whereas TLnA (loading: -0.931) exhibited a decrease. Time points formed a sequential trajectory along PC1, indicating progressive compositional changes, with PUFA strongly negatively correlated with time (decreasing trend) and SFA/MUFA positively correlated (increasing trend). This PCA successfully captured the metabolic shifts in fatty acid profiles during the experimental period, with PUFA degradation and SFA/MUFA accumulation being the dominant patterns driving the temporal separation.

The results from linear regression, hierarchical cluster analysis, and principal component analysis are highly congruent. Together, they demonstrate that thermal degradation is dominated by a strong, time-dependent linear decrease in nutritionally valuable PUFAs, coupled with a relative increase in SFAs and MUFAs. Furthermore, all three methods consistently identify that *trans* fatty acid formation (TLA, TTFA) follows a more complex, non-linear pathway distinct from the primary degradation trend. This multi-methodological alignment provides robust validation for the proposed degradation model.

Conclusion

The study results revealed that octadecanoic acid is affected differently when high trans-soybean oil is heated to 180±5°C for eight hours. Heat causes LnA and LA to gradually and significantly drop, OA to increase, TOA and TLA to slightly but noticeably increase, TLnA to significantly decrease, and TTFA to non-significantly decrease. For consumers in Bangladesh, the primary health risk arises from the high initial TFA content in refined oil, not necessarily from a dramatic increase during typical home cooking. However, heating further degrades nutritionally beneficial PUFAs and alters the TFA profile. Regulatory focus should be on limiting TFA formation during industrial refining/deodorization. Public awareness should stress using oils with low initial TFA and avoiding excessive reheating.

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