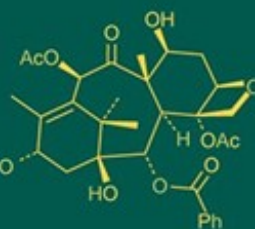
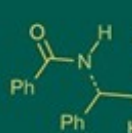


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Heat, reuse, and hidden risk: Oxidative and hydrolytic deterioration of commercial refined soybean oil under repeated thermal exposure

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ABSTRACT

Cooking oil reuse is common in Nigerian food-vending and household kitchens, where the same oil may be heated repeatedly across multiple frying sessions before disposal. Repeated thermal exposure can degrade oil quality in ways that are not visible to consumers, with potential implications for food quality and health. This preliminary study compared an unheated commercial refined soybean oil control with the same oil after ten 20-minute heating cycles at approximately 180 °C, monitored with a K-type thermocouple. Primary and secondary oxidation indices, lipid-peroxidation markers, hydrolytic indices, physicochemical properties, and an externally generated gas chromatography–mass spectrometry fatty-acid profile were evaluated. Peroxide value increased from 8.53 ± 0.13 to 12.31 ± 0.13 milliequivalents of oxygen per kilogram, representing a 44.2% increase. The *p*-anisidine value increased by 58.8% and total oxidation value by 51.9%. Conjugated-diene absorbance and thiobarbituric-acid-reactive substances expressed as malondialdehyde equivalents increased by 64.6% and 61.0%, respectively. Acid value and calculated free fatty acids increased by 53.7%, whereas iodine value decreased by 12.8%. Flash point declined by 4.9% and moisture content increased by 46.6%. These changes were directionally consistent across independent analytical indices, indicating oxidative and hydrolytic deterioration under the applied heating model. Because the heating treatment was performed as a single experimental run, the findings represent preliminary evidence and support the need for independently replicated studies of commercially available soybean oil.

Keywords: Soybean oil, Repeated heating, Thermal oxidation, Peroxide value, *P*-anisidine value, Fatty-acid composition

1. Introduction

Repeated use of cooking oil occurs in household and food-vending settings and can expose oils to successive periods of heat, oxygen and storage. Nigerian evidence indicates that cooking-oil reuse and mixing of residual oil with fresh oil occur among food vendors, although real-world practices vary in heating duration, food type, replenishment and storage conditions (Szabo *et al.*, 2022) [1]. Studies of frying oils from Port Harcourt and other Nigerian settings have likewise reported physicochemical deterioration associated with use and handling (Chen *et al.*, 2021; Kamisah *et al.*, 2012) [2, 3].

Soybean oil is rich in unsaturated fatty acids, particularly linoleic and alpha-linolenic acids. Carbon-carbon double bonds are susceptible to free-radical oxidation. During autoxidation and thermoxidation, lipid radicals react with oxygen to form hydroperoxides, which can subsequently decompose into aldehydes, ketones and other secondary products (ISO, 2024; ISO, 2020; ISO, 2016) [4, 5, 6]. Because primary products can accumulate and then decompose, no single oxidation index fully describes oil deterioration. Peroxide value (PV) and conjugated dienes reflect primary oxidation, whereas *p*-anisidine value (*p*-AV) and thiobarbituric-acid-reactive substances (TBARS) provide complementary information on secondary oxidation products (ISO, 2020; AOCS, 2017) [5, 7].

Repeated heating can also alter hydrolytic and bulk physicochemical properties. Acid value (AV) reflects titratable acidic constituents, while free fatty acids (FFA) may be calculated from AV when expressed as a specified fatty-acid equivalent. Iodine value (IV) provides an empirical measure of unsaturation. Changes in saponification value, refractive index, density, moisture and flash point may accompany deterioration, although their direction and magnitude depend on oil type and experimental conditions (ISO, 2016; Codex Alimentarius Commission, 2024) [6, 8]. Thermal processing can also alter *trans*-fatty-acid profiles, while repeatedly heated edible oils have been investigated in relation to potential cardiovascular effects (Soundararajan *et al.*, 2024; Bhat *et al.*, 2022) [9, 10].

For soybean oil, Codex CXS 210-1999 lists characteristic ranges including an iodine value of 124-139 g I₂/100 g and saponification value of 189-195 mg KOH/g; its supplementary quality factors include a maximum Acid value (AV) of 0.6 mg KOH/g and PV up to 10 meq active oxygen/kg for refined oils (Flores *et al.*, 2021) [11]. These benchmarks are useful for contextualizing baseline and heated-oil measurements, but they should not be interpreted as direct clinical safety thresholds.

The aim of this preliminary study was therefore to compare selected oxidation, lipid-peroxidation, hydrolytic and physicochemical indices of an unheated commercial refined soybean oil control with the same product after ten repeated 20-min heating cycles at 180 °C, and to describe the GC-MS fatty-acid profile and normalized degree of saturation and unsaturation.

2. Materials and methods

2.1 Oil sample and heating model

The experimental analyses were conducted at Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. A sealed 750 mL bottle of Laziz refined soya oil was purchased from a retail outlet in Okada, Edo State, Nigeria, where the product was displayed on a shelf at room temperature. The label identified the product as 100% refined soya oil fortified with vitamins A and E, manufactured by Apple and Pears Limited, Ogun State, Nigeria. The recorded batch number was 076LZ(A), the manufacturing date (MFD) was 17 March 2026, and the best-before date (BB) was 16 March 2028.

One 750 mL bottle was used for the repeated-heating experiment. The oil was transferred to an open stainless-steel pot and heated over a gas flame. Oil temperature was monitored with a portable K-type thermocouple digital probe thermometer (Hanna Instruments, Bedfordshire, UK). The gas flame was manually adjusted as required to maintain the oil at approximately 180 °C. For each heating cycle, the pot was first placed over the gas flame and the oil was allowed to reach 180 °C; the 20-min heating period was then timed from attainment of 180 °C. No food or water was intentionally added and no oil was removed or sampled between heating cycles. After each cycle, the oil was allowed to cool and remained in the stainless-steel pot at room temperature for approximately 24 h before the next cycle. Ten heating cycles were performed from 11 to 20 August 2026, giving a cumulative controlled heating period of 200 min at approximately 180 °C, excluding the time required to reach the target temperature.

A separate sealed 750 mL bottle of the same commercial product served as the unheated control and was kept at room temperature until analysis alongside the heated oil. After the final heating cycle, the heated oil was stored in a closed container at room temperature until laboratory analysis. The unheated control was designated U and the ten-cycle-heated oil H.

2.2 Primary and secondary oxidation products

Peroxide value (PV) was determined by iodometric titration using the laboratory procedure based on AOCS Official Method Cd 8-53 (Abeyrathne *et al.*, 2021) [12]. Briefly, 5.00 g oil was dissolved in 30 mL acetic acid-chloroform (3:2, v/v), reacted with 0.50 mL saturated potassium iodide, kept in the dark for 1 min, diluted with water and titrated with standardized 0.010 M sodium thiosulfate using starch near the endpoint. A reagent blank was included. Peroxide value (PV) was expressed as meq O₂/kg oil. Because the solvent system used was acetic acid-chloroform, the procedure is not described as ISO 3960:2017.

p-Anisidine value (*p*-AV) was determined according to ISO 6885:2016 using isooctane and *p*-anisidine reagent, with absorbance measured at 350 nm (Zhuang *et al.*, 2022) [13]. Total oxidation value (TOV) was calculated for each replicate as TOV = 2(PV) + *p*-AV.

2.3 Lipid peroxidation: conjugated dienes and thiobarbituric-acid-reactive substances

TBARS-derived malondialdehyde (TBARS-MDA) equivalents were determined by the external laboratory procedure. Approximately 1.0 g of oil was prepared using the laboratory solvent system and freshly prepared thiobarbituric acid reagent. Calibration standards were prepared from 1,1,3,3-tetramethoxypropane over 0.50-8.00 nmol/mL. Reaction mixtures were heated in a boiling-water bath under the laboratory-reported range of approximately 95-100 °C for approximately 15-30 min, cooled, centrifuged where required, and measured at approximately 532 nm against a reagent blank using a Shimadzu UV-1800 UV-Vis spectrophotometer. These ranges are reported exactly as documented by the laboratory; more precise run-specific conditions were not available. Results are therefore reported as TBARS-MDA equivalents rather than as chemically specific MDA.

Conjugated-diene formation was reported by the laboratory as absorbance at 233 nm (A₂₃₃) using the Shimadzu UV-1800. The laboratory record available for this manuscript contained the summary mean ± SD values but not the underlying replicate readings, solvent, oil concentration or cuvette path length. Because those experimental details were not recoverable, no unrecorded conditions were retrospectively assigned. Conjugated-diene measurements at approximately 232-234 nm are commonly used as a primary lipid-oxidation index (AOCS, 2017) [7].

2.4 Hydrolytic deterioration

Acid value (AV) was determined by titration in accordance with ISO 660:2020 (Zhang *et al.*, 2023) [14]. Approximately 2.00 g oil was dissolved in neutralized ethanol and titrated with standardized 0.100 M KOH using phenolphthalein. Acid value was expressed as mg KOH/g oil. Free fatty acid (FFA) was not independently measured; it was calculated from each Acid value replicate and expressed as oleic-acid equivalents using FFA (%) = AV × 0.503.

2.5 General physicochemical and fat characteristics

Saponification value (SV) was determined by alcoholic-KOH reflux followed by back-titration of excess alkali with standardized HCl. Iodine value was determined by the Wijs method using an AOCS/Codex-aligned oil-analysis procedure, approximately 0.20-0.50 g oil was dissolved in chloroform, reacted with 25.0 mL Wijs reagent in the dark for approximately 30 min, followed by potassium iodide, water and titration with 0.100 M sodium thiosulfate. ISO 3961:2024 [4] is the current ISO reference method for iodine value (Gharby *et al.*, 2025) [15]. Ester value was calculated for each replicate as SV - AV.

Unsaponifiable matter was determined after saponification with alcoholic KOH, petroleum-ether extraction, washing, solvent evaporation and gravimetric determination of the residue. Specific gravity was measured using a 25 mL glass pycnometer and refractive index using an ATAGO Abbe refractometer after laboratory temperature equilibration. The exact measurement temperatures were not recorded in the available laboratory documentation and are therefore acknowledged as a methodological limitation. Moisture was determined by oven drying at approximately 105 °C to constant mass. Flash point was measured using a Pensky-Martens closed-cup instrument; the specific standard designation used by the laboratory was not documented.

2.6 Fatty-acid composition and degree of unsaturation

2.6.1 Gas chromatography-mass spectrometry fatty-acid composition

Fatty-acid profiling was performed externally at the Chemical Analysis Laboratory, Department of Chemical and Petroleum Engineering, Afe Babalola University, Ado-Ekiti (ABUAD), Ekiti State, Nigeria. The laboratory report described analysis on a Varian 3800/4000 GC-MS fitted with a VF-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness), a reported nitrogen carrier-gas flow of 1.0 mL/min, scan range m/z 40-800, 45-min

run time and NIST08 library searching. Sample 01 was confirmed as H (Heated) and Sample 02 as U (Unheated).

The laboratory supplied one chromatographic profile per condition. The reported fatty-acid peak percentages were tabulated descriptively for the unheated and heated oils, and the characteristic Codex soybean-oil fatty-acid ranges were retained in Table 4 as reference values (Flores *et al.*, 2021) [11]. The heated profile had a reported total peak area of 105.54% before normalization; therefore, the degree-of-saturation/unsaturation indices were calculated from values normalized to 100% within each condition. The GC-MS results are reported descriptively and no inferential statistical test was applied to the single chromatographic profile obtained for each condition.

2.6.2 Normalized degree of saturation/unsaturation

Gas chromatography–mass spectrometry-identified fatty acids were classified as saturated fatty acids, monounsaturated fatty acids or polyunsaturated fatty acids. Total saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids and total unsaturated fatty acids were calculated after normalization of the reported fatty-acid percentages to 100%. Unsaturated-to-saturated fatty-acid and polyunsaturated-to-saturated fatty-acid ratios were calculated from the normalized totals. The directly measured iodine value was retained as the principal physicochemical index of unsaturation.

2.7 Statistical and data analysis

For laboratory endpoints with replicate observations, results are presented as mean $\pm$ standard deviation (SD) of three analytical determinations ($n = 3$). The heating treatment was conducted as one experimental run for the heated condition, with a separate unheated control oil; consequently, the triplicate analytical measurements quantify within-sample analytical repeatability and do not constitute three independently replicated heating experiments. Inferential tests such as *t*-tests or ANOVA were therefore not applied. Percent change was calculated from condition means. The GC-MS dataset comprised one chromatographic profile per condition and was treated descriptively only.

3. Results

Across every oxidative, hydrolytic and physicochemical index measured, repeated heating produced a change in the same direction predicted by thermal oxidation, and the magnitude of change was broadly consistent across independent, mechanistically distinct assays. Primary oxidation rose sharply: peroxide value increased by 44.2% and conjugated-diene absorbance by 64.6% (Tables 1–2). Secondary oxidation products tracked this rise, with *p*-anisidine value up 58.8% and TBARS-malondialdehyde equivalents up 61.0%, and total oxidation value, which combines the primary and secondary indices, rising by 51.9%. Hydrolytic deterioration followed the same pattern: acid value and its derived free fatty acid content each rose by 53.7%, alongside a 46.6% increase in moisture content, consistent with progressive hydrolysis and volatile loss during heating. Bulk physicochemical properties shifted accordingly: iodine value fell by 12.8%, flash point by 4.9%, and saponification and ester values declined modestly, while saponifiable matter rose by 28.0%. The externally generated GC-MS fatty-acid profile is reported descriptively (Table 4) because the values for several fatty acids fall outside the Codex characteristic range for soybean oil; within that constraint, the normalized data showed a relative increase in saturated fatty acids and a corresponding decrease in the unsaturated fraction after heating, in the same direction as the Wijs iodine-value result. Taken together, the convergence of independent primary-oxidation, secondary-oxidation, hydrolytic and physicochemical indices provides internally consistent evidence of heating-associated deterioration under the conditions tested.

3.1 Primary and secondary oxidation indices

Results with replicate observations were reported as mean $\pm$ SD of three analytical determinations. All measured oxidation indices were higher after ten heating cycles. PV increased by 44.2%, *p*-AV by

58.8%, and TOV by 51.9% relative to the unheated control (Table 1).

3.2 Lipid peroxidation

Conjugated-diene absorbance increased by 64.6%, while TBARS-MDA equivalents increased by 61.0% after repeated heating (Table 2), providing directionally consistent evidence of increased lipid oxidation.

3.3 Hydrolytic and physicochemical fat characteristics

AV increased by 53.7% and calculated FFA by 53.7%, whereas IV decreased by 12.8% (Table 3). These baseline values were considered when interpreting the heating-associated changes.

3.4 Fatty-acid composition and degree of unsaturation

3.4.1 Gas chromatography–mass spectrometry fatty-acid composition reported for unheated and ten-cycle-heated soybean oil

The externally generated chromatograms are shown in Figures 1 and 2. The raw reported percentages summed to 99.99% for U (Unheated) and 105.54% for H (Heated) (Table 4). The fatty-acid results were presented descriptively as reported by the external laboratory. For calculation of the degree of saturation and unsaturation, the reported fatty-acid percentages were normalized to 100% within each condition.

3.4.2 Normalized degree of saturation and unsaturation

The laboratory-reported fatty-acid percentages were grouped as SFA, MUFA and PUFA and normalized to 100% within each condition. The resulting indices were shown in Table 5. After normalization, SFA increased from 33.93% in U to 35.76% in H, while MUFA changed slightly from 24.58% to 24.51%. PUFA decreased from 41.49% to 39.72%, and total UFA decreased from 66.07% to 64.24%. Correspondingly, the UFA/SFA ratio decreased from 1.95 to 1.80 and the PUFA/SFA ratio from 1.22 to 1.11.

These normalized values described the relative distribution of saturated and unsaturated fatty-acid classes after rescaling each GC-MS profile to 100%. They were presented as descriptive compositional indices for U and H and were not subjected to inferential statistical testing because one chromatographic profile was available for each condition.

4. Discussion

The principal observation was a coherent increase in several oxidation-related indices after ten repeated heating cycles. PV rose from 8.53 ± 0.13 to 12.31 ± 0.13 meq O₂/kg, while *p*-AV, TOV, conjugated dienes and TBARS-MDA also increased. Taken together, these changes are compatible with greater formation of primary oxidation products and their conversion to secondary products during repeated thermal exposure. This multi-index approach is preferable to interpreting PV alone because hydroperoxides are transient and can decompose as oxidation progresses (ISO, 2024; ISO, 2020; AOCS, 2017) [4, 5, 7].

The magnitude and direction of the PV change are consistent with previous experimental work on repeatedly heated soybean oil. Repeated frying/heating studies have reported increasing PV with repeated use, including soybean-oil models in which five heating cycles produced PV values around 13–17 meq/kg depending on the fried food matrix (Okidhika *et al.*, 2023) [16]. In the present model, the oil was maintained at approximately 180 °C for 20 min per cycle using gas-flame adjustment guided by a K-type thermocouple, and no food or added water was present; differences in heating design should therefore be considered when comparing studies.

Baseline quality requires particular attention. Codex lists a supplementary maximum AV of 0.6 mg KOH/g and PV up to 10 meq/kg for refined oils (Flores *et al.*, 2021) [11]. Although the unheated PV of 8.53 meq/kg was below that value, its AV of 2.87 mg KOH/g was higher than the refined-oil supplementary value. In addition, the measured IV of 78.80 g I₂/100 g was below the Codex characteristic soy

bean-oil range of 124-139 g I₂/100 g (Flores *et al.*, 2021) [11]. Nigerian market surveys have shown that physicochemical quality can vary among commercially available oils and with storage/handling (Chen *et al.*, 2021; Kamisah *et al.*, 2012) [2, 3]. These baseline characteristics were therefore considered when interpreting the changes observed after heating.

AV increased further to 4.42 ± 0.06 mg KOH/g after heating, with calculated FFA increasing correspondingly. Because FFA was mathematically derived from AV, these are related rather than independent findings. Hydrolysis is often promoted by water, but no water or food was intentionally added here. The increase may reflect acidic products generated during repeated thermal exposure and possible environmental moisture uptake during cooling and storage. The measured moisture content increased from $0.19 \pm 0.02\%$ to $0.28 \pm 0.02\%$, although the mechanism responsible was not directly tested.

IV decreased by approximately 12.8%, from 78.80 ± 0.37 to 68.74 ± 0.39 g I₂/100 g. This direction of change is compatible with alter-

ation of double-bond-containing lipids during thermal oxidation. The current ISO iodine-value standard notes that IV can be determined directly and can also be estimated from fatty-acid composition under specified conditions (Gharby *et al.*, 2025) [15]. In the present study, the directly measured Wijs IV was retained as the principal physicochemical index of unsaturation, while the GC-MS-derived SFA, MUFA, PUFA and UFA indices were reported descriptively.

SV decreased modestly, and the recalculated ester value decreased from 185.36 ± 0.62 to 177.65 ± 0.59 mg KOH/g. These changes may reflect alteration of the glyceride mixture and accumulation or loss of components during heating. Unsaponifiable matter increased from $1.24 \pm 0.03\%$ to $1.59 \pm 0.03\%$. Because unsaponifiable matter is expressed relative to sample mass, the observed increase may reflect a change in the relative contribution of nonsaponifiable constituents after repeated heating.

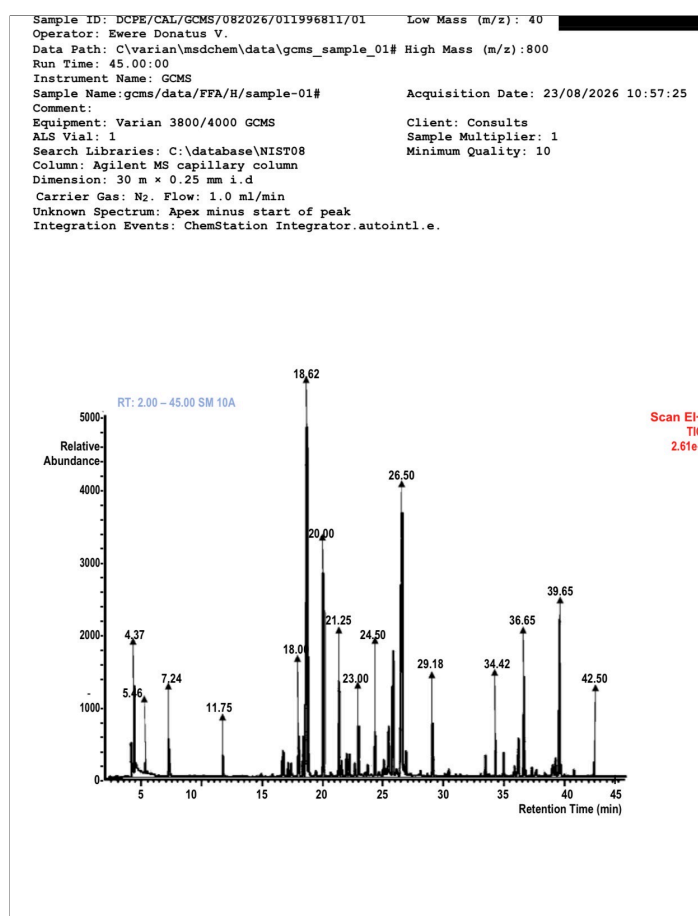


Figure 1. GC-MS total-ion chromatogram supplied for the heated oil (H; Sample 01).

Specific gravity increased slightly from 0.917 ± 0.001 to 0.922 ± 0.001 , while refractive index decreased from 1.4686 ± 0.0004 to 1.4652 ± 0.0004 . Physicochemical responses to heating depend on oil composition and thermal conditions (ISO, 2016; Codex Alimentarius Commission, 2024) [6, 8]. Flash point also decreased from 219.33 ± 1.53 to 208.67 ± 1.53 °C, which may be associated with the formation of more volatile degradation products during repeated heating.

The GC-MS profile provided a descriptive comparison of the fatty-acid composition reported for U and H. Following normalization, SFA increased from 33.93% to 35.76%, MUFA changed from 24.58% to 24.51%, PUFA decreased from 41.49% to 39.72%, and total UFA decreased from 66.07% to 64.24%. The UFA/SFA and PUFA/SFA ratios also decreased from 1.95 to 1.80 and from 1.22 to 1.11, respectively. These normalized indices indicate a shift in the relative distribution of the reported fatty-acid classes after repeated heating and are interpreted descriptively because one GC-MS profile was obtained for each condition. Repeated heating has been reported to alter fatty-acid

composition in plant-based cooking oils, with the extent of change depending on oil type and heating conditions (Oladunjoye *et al.*, 2024) [17].

This study should be interpreted as a preliminary controlled heating experiment. The heating treatment was performed as one experimental run using one 750 mL oil sample, while laboratory measurements were repeated three times where replicate analytical data were available. Thus, the reported mean $\pm$ SD values describe analytical repeatability within each oil condition rather than variability among independently repeated heating experiments. Oil temperature was monitored with a K-type thermocouple and maintained at approximately 180 °C by manual adjustment of the gas flame. The unheated control remained sealed, whereas the heated oil was exposed to the open stainless-steel pot during heating and cooling. The model did not reproduce all features of commercial frying because no food, water, crumbs or oil replenishment were introduced. Total polar compounds, polymerized triacylglycerols, tocopherols, colour and viscosity were

not measured, although total polar compounds are particularly important in evaluating frying-oil deterioration (Emelike *et al.*, 2020) [18]. Exact conjugated-diene assay preparation details and some instrument-specific conditions for the physicochemical measurements were

not available in the laboratory documentation. The cumulative 200 min at approximately 180 °C represents the defined laboratory exposure used in this study and should not be equated directly with a specific commercial-vendor reuse duration.

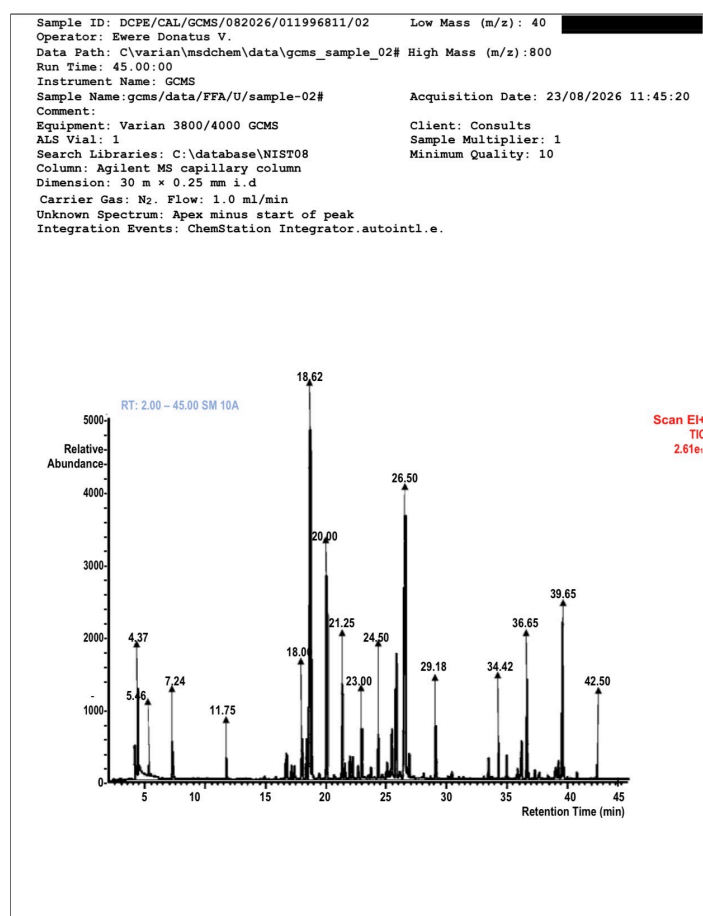


Figure 2. GC-MS total-ion chromatogram supplied for the unheated control (U; Sample 02).

Future studies should include independently replicated heating experiments, standardized vessel geometry and oil depth, matched storage conditions, intermediate sampling across cycles, and complementary deterioration indices such as total polar compounds, viscosity, colour, tocopherols and polymerization products.

5. Conclusion

This study provides coherent, multi-index evidence that repeated heating of commercial refined soybean oil under a controlled laboratory model, ten cycles of 20 minutes at approximately 180 °C, drives measurable oxidative and hydrolytic deterioration. Peroxide value, *p*-anisidine value, total oxidation value, conjugated dienes and TBARS-malondialdehyde equivalents rose in tandem, while acid value, free fatty acids, moisture and unsaponifiable matter increased and iodine value, flash point and saponification value declined. The convergence of these independent, mechanistically distinct indices, rather than any single measurement, is what makes the evidence of deterioration credible: primary oxidation products accumulated and were converted to secondary products, hydrolysis progressed, and the physicochemical character of the oil shifted measurably away from its unheated baseline. Critically, the heated peroxide value exceeded the Codex advisory maximum for refined oils, a finding with direct relevance to food-vending and household practices in which oil is reused across multiple heating sessions. At the same time, because this heating exposure was carried out as a single experimental run, with the reported precision reflecting analytical repeatability rather than independent replication, these findings should be read as preliminary rather than conclusive. They nonetheless establish a clear, reproducible direction

of change and a practical basis for larger, independently replicated studies, incorporating total polar compounds, tocopherol retention and standardized heating protocols, that can translate this laboratory evidence into concrete guidance on safe reuse limits for soybean oil in the Nigerian food-vending context.

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Author contributions

All authors contributed to the development and completion of the study through combinations of conceptual input, resources, financial support, scientific guidance and manuscript development. Onovughe Faith Avwersuoghene contributed to investigation, data compilation, analysis and drafting of the manuscript. Helen Kwipnchep Njoya contributed to supervision, scientific guidance and critical review. Esumai Ifeoma Victory and Osimen-Eboziegbe Uche Christina contributed resources, scientific input and review/editing of the manuscript. All authors reviewed and approved the final manuscript.

Data availability

The analytical data supporting the findings of this study are included in the manuscript and supplementary tables. Additional original laboratory records available to the authors may be provided on reasonable request, subject to institutional and laboratory restrictions.

Conflict of interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary Table S1: Verified replicate-level analytical data. Replicate observations are analytical determinations from the U and H conditions and are the basis for the reported mean $\pm$ SD values; they are not independent heating runs.

Tables

Table 1. Primary and secondary oxidation indices. Values are mean $\pm$ SD of three analytical determinations ($n = 3$). TOV was calculated for each replicate as $2(PV) + p-AV$.

Parameter	Unheated control (U)	Ten-cycle heated (H)	Change
Peroxide value (PV) (meq O ₂ /kg)	8.53 $\pm$ 0.13	12.31 $\pm$ 0.13	+44.2%
<i>p</i> -Anisidine value (<i>p</i> -AV)	18.88 $\pm$ 0.24	29.97 $\pm$ 0.23	+58.8%
Total oxidation value (TOV)	35.94 $\pm$ 0.49	54.58 $\pm$ 0.48	+51.9%

Table 2. Lipid-peroxidation indices. Values are mean $\pm$ SD. Conjugated-diene values are the laboratory-supplied summary values; underlying replicate readings were unavailable.

Parameter	Unheated control (U)	Ten-cycle heated (H)	Change
Conjugated dienes (A ₂₃₃)	0.684 $\pm$ 0.012	1.126 $\pm$ 0.018	+64.6%
TBARS-MDA (nmol/mL)	2.90 $\pm$ 0.03	4.67 $\pm$ 0.04	+61.0%

Table 3. Hydrolytic and physicochemical fat characteristics. Values are mean $\pm$ SD of three analytical determinations ($n = 3$). FFA and ester value were recalculated from replicate-level AV and SV data. Percent change was calculated from means.

Characteristic	U (Unheated)	H (Heated)	Change
Acid value (AV) (mg KOH/g)	2.90 $\pm$ 0.03	4.42 $\pm$ 0.06	+53.7%
Free fatty acid (FFA) (% oleic acid)	1.445 $\pm$ 0.018	2.222 $\pm$ 0.030	+53.7%
Saponification value (SV) (mg KOH/g)	188.23 $\pm$ 0.65	182.07 $\pm$ 0.65	-3.3%
Iodine value (IV) (g I ₂ /100 g)	78.80 $\pm$ 0.37	68.74 $\pm$ 0.39	-12.8%
Ester value (mg KOH/g)	185.36 $\pm$ 0.62	177.65 $\pm$ 0.59	-4.2%
Unsaponifiable matter (%)	1.24 $\pm$ 0.03	1.59 $\pm$ 0.03	+28.0%
Specific gravity	0.917 $\pm$ 0.001	0.922 $\pm$ 0.001	+0.5%
Refractive index	1.4686 $\pm$ 0.0004	1.4652 $\pm$ 0.0004	-0.2%
Moisture (%)	0.19 $\pm$ 0.02	0.28 $\pm$ 0.02	+46.6%
Flash point (°C)	219.33 $\pm$ 1.53	208.67 $\pm$ 1.53	-4.9%

Table 4. Laboratory-reported GC-MS fatty-acid composition of unheated and ten-cycle-heated soybean oil, with characteristic Codex soybean-oil fatty-acid ranges shown for reference. ND = non-detectable in the Codex table.

Fatty acid	Class	U(Unheated) (wt. %)	H(Heated) (wt. %)	Codex Soybean range (%)
Octanoic acid (C ₈ :0)	SFA	7.36	6.33	ND
<i>n</i> -Decanoic acid (C ₁₀ :0, capric)	SFA	3.14	3.05	ND
Dodecanoic acid (C ₁₂ :0, lauric)	SFA	1.17	1.60	ND
Tetradecanoic acid (C ₁₄ :0, myristic)	SFA	2.82	2.77	ND-0.1
<i>n</i> -Hexadecanoic acid (C ₁₆ :0, palmitic)	SFA	2.97	3.58	8.0-13.5
Octadecanoic acid (C ₁₈ :0, stearic)	SFA	1.62	1.58	2.0-5.4
Eicosanoic acid (C ₂₀ :0, arachidic)	SFA	3.37	3.93	0.1-0.6
Docosanoic acid (C ₂₂ :0, behenic)	SFA	2.36	3.14	ND-0.7
Lignoceric acid (C ₂₄ :0)	SFA	9.11	11.77	ND-0.5
Palmitoleic acid (C ₁₆ :1)	MUFA	11.57	12.63	ND-0.2

(continued on next page)

Fatty acid	Class	U(Unheated) (wt. %)	H(Heated) (wt. %)	Codex Soybean range (%)
Oleic acid (C ₁₈ :1)	MUFA	5.99	5.47	17-30
cis-11-Eicosenoic acid (C ₂₀ :1)	MUFA	1.02	1.31	ND-0.5
Erucic acid (C ₂₂ :1)	MUFA	6.00	6.46	ND-0.7
Linoleic acid, 9,12-C18:2 (Z,Z)	PUFA	2.95	3.37	48.0-59.0
Linolenic acid, 9,12,15-C18:3 (Z,Z,Z)	PUFA	19.55	19.46	4.5-11.0
C20:4 fatty acid (tentative assignment)	PUFA	18.99	19.09	Not characteristic
Total reported area		99.99	105.54	100 (expected total)

Table 5. Normalized degree of saturation and unsaturation of unheated and ten-cycle-heated soybean oil. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = total unsaturated fatty acids. Values are descriptive normalized indices derived from one GC-MS profile per condition.

Normalized index	Unheated (U)	Ten-cycle heated (H)	Observed change
Total Saturated fatty acid (SFA) (%)	33.93	35.76	+1.83 percentage points
Total Monounsaturated fatty acid (MUFA) (%)	24.58	24.51	-0.07 percentage points
Total Polyunsaturated fatty acid (PUFA) (%)	41.49	39.72	-1.77 percentage points
Total Unsaturated fatty acid (UFA) (%)	66.07	64.24	-1.83 percentage points
UFA/SFA ratio	1.95	1.80	-7.7%
PUFA/SFA ratio	1.22	1.11	-9.0%

Parameter	Unit	U1	U2	U3	H1	H2	H3
Peroxide value (PV)	meq O ₂ /kg	8.42	8.67	8.51	12.18	12.43	12.31
<i>p</i> -Anisidine value (<i>p</i> -AV)	-	18.64	19.12	18.87	29.76	30.21	29.94
Total oxidation value (TOV) (calculated)	-	35.48	36.46	35.89	54.12	55.07	54.56
TBARS-MDA	nmol/mL	2.88	2.94	2.89	4.63	4.71	4.68
Acid value (AV)	mg KOH/g	2.84	2.91	2.87	4.36	4.48	4.41
Free fatty acid (FFA) (calculated)	% oleic acid	1.429	1.464	1.444	2.193	2.253	2.218
Saponification value (SV)	mg KOH/g	187.6	188.9	188.2	181.4	182.7	182.1
Iodine value (IV)	g I ₂ /100 g	78.42	79.16	78.81	68.35	69.12	68.74
Ester value (calculated)	mg KOH/g	184.76	185.99	185.33	177.04	178.22	177.69
Unsaponifiable matter	%	1.21	1.27	1.24	1.56	1.61	1.59
Specific gravity	-	0.916	0.918	0.917	0.921	0.923	0.922
Refractive index	-	1.4682	1.4690	1.4686	1.4648	1.4655	1.4652
Moisture	%	0.18	0.21	0.19	0.27	0.30	0.28
Flash point	°C	218	221	219	207	210	209

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