# *Original Research Article*

# Development and Validation of a Paper-Based Strip for Detecting Oil Degradation and Trans-Fat Formation in Repeatedly Heated Palm Oil

**ABSTRACT**

**Aims:** This study aims to create a simple and quick test using a paper-based strip to check how much palm oil degrades when it’s used repeatedly for frying.

**Study Design:** An experimental study combining traditional lab techniques with the newly developed strip method.

**Place and Duration of Study:** Department of Food Science and Nutrition, The American College, Madurai, Tamil Nadu, India.

**Methodology:** Palm oil was heated for up to 8 hours. Samples were collected every 2 hours and analyzed using UV-Vis, fluorescence spectroscopy, and GC-MS. The paper-based strip, infused with color-changing chemicals and silver ions, was tested for its ability to detect oil degradation.

**Results:** The natural antioxidants in palm oil, like carotenoids, broke down with time, causing a clear drop in fluorescence. Harmful compounds such as trans fats and oxidized sterols were found using GC-MS. UV-Vis readings showed increasing oxidation levels. The paper strips changed color depending on the oil’s condition, offering a clear visual indicator of oil quality.

**Conclusion:** The paper strip method is fast, affordable, and easy to use. It provides a practical way to check palm oil quality in kitchens without needing complex lab tools.

**Keywords:** Palm oil, paper-based strip, oil degradation, trans-fat, GC-MS, fluorescence spectroscopy

# 1. INTRODUCTION

Palm oil is widely used because of its great oxidative stability, low cost, and excellent frying properties. It has a balanced ratio of saturated and unsaturated fatty acids and is naturally high in antioxidants such as tocopherols and carotenoids. Despite these benefits, the repeated use of palm oil in frying might cause a gradual deterioration in nutritional quality and the development of potentially hazardous chemicals (Wen *et al.,* 2023).

One of the primary problems of reused oils is the buildup of free fatty acids, trans fats, and polymerized triglycerides, all of which have been associated to chronic illnesses such as cardiovascular disease, metabolic syndrome, and certain malignancies. Heating oils over their smoke temperatures promotes oxidative and thermal deterioration, resulting in off-flavors, increased viscosity, and darker coloration—all symptoms of poor oil quality (Folahan *et al.,* 2023).

While laboratory procedures like as gas chromatography and UV-Vis spectroscopy are gold standards for identifying oil deterioration, they are impracticable for frequent inspections in high-speed food situations. As a result, there is an increasing demand for simple, portable, and inexpensive testing technologies that allow for real-time monitoring of oil quality. This work presents and evaluates a unique paper-based strip for assessing the quality of palm oil after repeated heating. This strip, which combines visual signals with colorimetric chemistry, provides a new, field-friendly approach for early detection of oil deterioration (Spangenberg & Lantos, 2024).

# 2. MATERIAL AND METHODS

# 2.1 Sample Collection and Preparation

Palm oil was bought from a local shop and heated for 0, 2, 4, 6, and 8 hours to mimic typical frying conditions. Samples were collected at each stage (Zhang *et al.,* 2018).

# 2.2 Visual Observation

As the oil was heated longer, it turned from light yellow to darker orange and became thicker. These visual cues are early signs of oil degradation (Zhang *et al.,* 2018).

# 2.3 Fluorescence Spectroscopy

Fluorescence spectroscopy was conducted using a xenon lamp with excitation and emission wavelength ranges of 240–475 nm and 250–600 nm, respectively. A photomultiplier was set to a voltage of 850 V to ensure optimal detection. 23 samples one by one each, vigorously shaken to ensure homogeneity, was directly poured into a quartz cuvette for measurement. The cuvettes were thoroughly rinsed with hexane between sample runs to prevent cross-contamination. Data were collected in increments of 2 nm for emissions and 5 nm for excitation, with an integration time of 0.1 seconds (Wang *et al.,* 2019).

# 2.4 GC-MS Analysis

The 23 oil samples were collected in all test tube and were dissolved in 100 μL of hexane, and 1 μL of each sample was injected five times into a gas chromatograph. The separation and quantification of samples were carried out using a BPX-70 fused silica capillary column, following an optimized temperature program. The column temperature was initially set at 100°C and gradually increased to a final temperature of 260°C. The analysis was conducted with a carefully controlled carrier gas flow rate of 1 mL/min (Zhang *et al.,* 2020).

# 2.5 UV-Vis Spectroscopy

UV-Visible spectrophotometer equipped with a deuterium lamp for UV and a tungsten lamp for visible light was employed to analyze the 23 oil samples. Measurements were conducted in quartz cuvettes, with a 1 mL sample containing 0.9 mL hexane and 0.1 mL oil. Spectra were recorded across wavelengths of 200–800 nm at 1 nm intervals. For calibration, both sample and blank cells were initially filled with hexane, and the blank spectra were subtracted prior to analysis. All samples were analyzed in duplicate. (Saputera *et al.,* 2021).

# 2.6 Paper-Based Strip Development

The paper-based sensor was developed using **Whatman Grade 1 filter paper**, known for its uniform fiber distribution, chemical purity, and heat stability (tolerant up to 120°C). This makes it suitable for oil sample contact without thermal degradation or leaching. Strips were cut into 5 cm × 0.3 cm dimensions to ensure consistent reagent uptake and surface interaction.To enable selective detection of oxidized compounds, the strips were treated with **3,3′,5,5′-tetramethylbenzidine (TMB)** and **silver nitrate (AgNO₃)**:

* TMB acts as a chromogenic agent that produces a deep blue color upon oxidation.
* Silver ions (Ag⁺) oxidize TMB when not consumed by lipid peroxides or aldehydes in degraded oil.

The procedure:

1. Strips were immersed in 0.2 M sodium acetate buffer (pH 4.5) for 10 minutes to ensure chemical uniformity.
2. A 50 mM TMB ethanol solution was applied, followed by drying.
3. Oil samples were mixed with 10 mM AgNO₃ and incubated with the strips for 10 minutes.
4. Color change (blue intensity) was observed and recorded, with darker blue indicating lower degradation.

This mechanism exploits the competition between oxidants in the oil and the TMB reaction, enabling **visible detection of degradation** without instruments.(Zhang *et al.,* 2018).

# 3. RESULTS AND DISCUSSION

The longer palm oil was heated, the more it broke down—both visibly and chemically. It darkened in color and became thicker, hinting at oxidation and polymer buildup. These physical changes were supported by lab data (Fig. 1) (Ho *et. al,* 2021, Wazed M. A. *et. al,* 2021).

**Table No. 1 COMPARATIVE ANALYSIS OF PALM OIL SAMPLES**

|  |  |  |
| --- | --- | --- |
| **Heating Time** | **Observation** | **Interpretation** |
| **Control** | Light yellow | Fresh oil, minimal oxidation. |
| **0 hr Heated** | Light yellow | Initial heating, no visible change. |
| **2 hr Heated** | Slight yellow | Minor oxidation due to saturated fat content. |
| **4 hr Heated** | More yellow | Formation of primary oxidation products. |
| **6 hr Heated** | Yellow-orange,slightly viscous | Polymerization begins, breakdown of triglycerides. |

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**C**

**B**

**A**

**Fig. 1 - PALM OIL HEATED SAMPLE**

A – Control to 8 hr heated samples, B – Control Sample, C- 8 hr Sample

With increased heating duration (0–8 hours), palm oil turned from a light yellow to an intense orange-brown color and became noticeably more viscous. These physical cues—darkening and thickening—indicate polymerization and the accumulation of degradation products like oxidized triglycerides. These observations aligned with results from previous studies on thermal stress in edible oils (Wazed et al., 2021; Zhang et al., 2018).

Fluorescence spectroscopy indicated that as the oil was heated for an extended period of time, the intensity of its emissions decreased (Fig. 5). Fresh palm oil produced significant fluorescence signals, owing to its high concentration of carotenoids and tocopherols, which naturally release light when stimulated at certain wavelengths (330-350 nm excitation, 450-470 nm emission) (Fig. 2 & 3). After 4 hours of heating, there was a considerable drop in fluorescence, and by the eighth hour, the intensity had decreased by more than 70% (Fig. 4). This fast decline reflects the depletion of endogenous antioxidants, which are essential to the oil's protective effects. This deterioration is consistent with the predicted breakdown under thermal oxidative stress, indicating lower nutritional quality and a higher likelihood of hazardous byproduct production (Sangjan *et. al,*., 2022).

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**Fig. 2 - EMISSION SPECTRA Fig. 3 - EXCITATION SPECTRA**

 

**Fig. 4 - RELATIVE CONCENTRATION Fig. 5 – FLUORESCENCE SPECTROSCOPY ANALYSIS**

**Table No. 2 FLUORESCENCE SPECTROSCOPY ANALYSIS OF PALM OIL SAMPLES**

|  |  |
| --- | --- |
| Aspect | Palm Oil |
| Key Antioxidants | Carotenoids, tocopherols |
| Control Sample Fluorescence | Strong signal dominated by carotenoids and tocopherols |
| Effect of Heating on Fluorescence | Significant decline after 4–6 hours |
| Chemical Changes | Degradation of carotenoids and tocopherols, formation of harmful oxidation products (e.g., trans fats) |
| Health Implications | Reduced antioxidant capacity, formation of toxic compounds linked to inflammation and chronic diseases |
| Major By-Products Formed | Aldehydes, ketones, trans fats |
| Recommendations | Minimize heating to preserve nutritional quality and prevent harmful compound formation |

Gas chromatography-mass spectrometry (GC-MS) revealed precise information on the chemical changes occurring in the oil. Fresh palm oil included mostly beneficial fatty acids and sterols. (Fig. 6 & 7) The GC-MS spectra revealed the development of hazardous chemicals, such as:

* 2,3-Dihydroxypropyl elaidate – a trans-fat derivative formed through isomerization.
* Beta-sitosterol acetate – a marker of sterol oxidation.
* Acrolein and glycidyl esters – toxic compounds associated with DNA damage and inflammation.

Oxidized hydrocarbons and breakdown products of squalene and phytosterols also increased significantly. These chemicals are not only indicators of deterioration, but they have also been related to long-term health issues such as cancer and atherosclerosis. The presence of these compounds corroborated the paper strip's visual alterations and demonstrated that it can efficiently detect chemical deterioration in palm oil (Syafri *et. al,* 2024).



**Fig. 6 - GC-MS ANALYSIS OF PALM OIL**

**Fig. 7 - PEAK AREA ANALYSIS OF PALM OIL**

**Table No. 3 GC-MS ANALYSIS OF PALM OIL SAMPLES**

|  |  |
| --- | --- |
| **Parameter** | **Palm Oil** |
| **Key Compounds Formed** | Beta-sitosterol acetate, 2,3-dihydroxypropyl elaidate, squalene oxidation |
| **Carcinogenic Compounds** | Glycidyl palmitate, Acrolein |
| **Mutagenic Compounds** | 2-Hydroxy-1-(hydroxymethyl)ethyl ester |
| **Trans Fats** | 2,3-Dihydroxypropyl elaidate, cis-13-octadecenoic acid |
| **Oxidation Products** | Oxidized phytosterols (oxysterols), free radicals |
| **Harmful Byproducts** | Acrolein, aldehydes, Campesterol |

UV-Vis spectra revealed gradual changes in absorbance patterns, notably in the 240-280 nm range—associated with primary oxidation products such as hydroperoxides and conjugated dienes. The control sample showed a modest absorbance, indicating no oxidative damage. However, with each heating cycle, the absorbance readings increased progressively. After 6 hours, peaks in the 270-300 nm range appeared, indicating secondary oxidation products such as aldehydes and ketones. These alterations verified oxidation while also indicating possible taste, fragrance, and health effects connected with the oil's ongoing usage (Fig. 8) (Deekshitha *et. al,.,* 2024).

**Table No. 4 UV-VIS SPECTROSCOPY ANALYSIS OF PALM OIL SAMPLES**

|  |  |
| --- | --- |
| **Parameter** | **Palm Oil** |
| **Wavelength Range (Analyzed)** | 200–400 nm |
| **Primary Absorbance Region** | 240–280 nm |
| **Key Peaks and Absorbance Behavior** | Peaks at 230–250 nm (dienes) and 250–270 nm (trienes). |
| **Trend with Heating** | Gradual increase in absorbance from POC1 to POT4 (2–8 hrs). |
| **Primary Oxidation Products** | Hydroperoxides, dienes, and trienes (240–280 nm). |
| **Secondary Oxidation Products** | Aldehydes, ketones, and trans fatty acids. |
| **Absorbance Beyond 300 nm** | Minimal absorbance beyond 300 nm. |
| **Health Implications** | Accumulation of toxic compounds like acrylamides and PAHs with prolonged use. |
| **Overall Observations** | Progressive degradation, producing hazardous trans fats and oxidation products. |



**Fig. 8 - UV-VIS SPECTROSCOPY ANALYSIS OF PALM OIL**

The colorimetric paper strip exhibited a clear, visually interpretable response: **a deep blue color in fresh oil samples** that faded with increasing degradation. The reaction relies on TMB oxidation by silver ions in the presence of lipid peroxides and aldehydes, which compete for silver and limit its ability to trigger the blue reaction.

* In **fresh oil**, antioxidants neutralize reactive compounds, allowing silver to oxidize TMB fully, resulting in a **dark blue** strip.
* In **degraded oil**, lipid peroxides and aldehydes consume available silver ions, reducing TMB oxidation and yielding a **pale blue or colorless** strip.

This color change correlates with:

* **Fluorescence decay** (due to antioxidant depletion),
* **GC-MS detection** of trans fats and oxidized sterols,
* **UV-Vis absorbance increases** associated with oxidation.

A simple visual table or color chart (to be included in final manuscript) could serve as a reference scale for users to interpret oil quality based on strip response.



**Fig. 9 - PAPER BASED STRIP OF PALM OIL**

A – Control Sample, B – 8 hr Sample

The results from conventional lab techniques and the strip-based method showed strong agreement, confirming the strip’s potential as a surrogate tool for detecting oil spoilage. Unlike spectroscopic or chromatographic tools that require lab settings, the strip offers an easy-to-use, low-cost, and **field-deployable** alternative. This can be particularly beneficial for small-scale food vendors, households, or institutional kitchens with limited access to laboratory infrastructure.

Further improvements in the **strip’s sensitivity**, **color stability**, and **packaging** can enable large-scale production and commercialization for real-time oil quality monitoring.

# 4. CONCLUSION

When palm oil is used regularly, it degrades chemically and physically, producing hazardous compounds such as trans fats, oxidized sterols, and aldehydes. These chemicals offer serious health hazards, including inflammation, oxidative stress, and carcinogenic potential. This study indicated that a paper-based strip might be a useful, quick, and low-cost instrument for identifying oil deterioration.

 The strip not only indicates the oil's deterioration condition by simple color changes, but it also correlates well with advanced laboratory methods such as UV-Vis, fluorescence spectroscopy, and GC-MS. Its simplicity and real-time feedback make it particularly helpful in home kitchens, small food establishments, and underdeveloped countries where analytical equipment is scarce.

Further enhancements to strip sensitivity, stability, and packaging may lead to commercialization and greater application in food safety monitoring. Overall, this invention has the potential to help consumers and food handlers select safer and healthier oil choices.

Altogether, these results show that the paper strip is not just a gimmick—it reliably tracks oil spoilage in real time. It's a cost-effective tool, especially useful in places where regular lab testing isn’t possible.

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