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Research Article

Assessment of chemical characteristics of repeatedly fried edible oil

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Abstract: The repeated use of edible oils to fry food has been a long-standing practice. Unfortunately, many people, especially in developing countries, are unaware of the health risks associated with consuming such oils. In this study, palm olein oil, the most commonly used edible oil in Nigeria, was used to fry fish three times, and ten times. Samples of fresh (non-fried), three-times fried, and ten-times fried oil were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The analysis revealed that oleic acid (9-octadecenoic acid) was the primary chemical constituent in fresh palm olein oil, accounting for 33.48% of its composition. Other components in fresh palm olein oil included hexadecanoic acid (7.08%), 9,12-octadecanoic acid (8.16%), and benzene derivatives such as nonyl benzene (3.49%) and octyl benzene (2.895%). The three times fried oil contained 17.08% oleic acid, along with cholesterol, 2-Ethylacridine, 2,4,6-Cycloheptatrien-1-one, and Cyclotrisiloxane, hexamethyl. The ten times fried oil contained 14.15% oleic acid, 3.40% cholesterol, 11.69% 2-ethylacridine, 19.15% 2,4,6-Cycloheptatrien-1-one, 15.54% 1,4-Bis-(trimethylsilyl)-benzene, and 23.08% 1,2-Benzisothiazol-3-amine. The findings of this study suggest that with an increase in the number of frying times, the nutritional quality of the oil decreases, and more cyclic aromatic hydrocarbons are generated. These hydrocarbons have been linked to the development of chronic diseases, including cancer and cardiovascular diseases.

Keywords: Edible oil, Repeatedly fried, Fish, Gas Chromatography Mass Spectrophotometer, Chemical constituents

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Introduction

The relationship between diet and human diseases has long been established. Hippocrates (460 B.C.) first reported a strong positive association between food consumption and human diseases [1]. Regular consumption of healthy foods promotes good health, while poor dietary choices can contribute to or worsen a wide range of diseases, including cardiovascular disease, cancer, and diabetes [2]. Unsafe food can also cause infectious diseases through food poisoning [3].

Reports show that unsafe food containing harmful toxins or toxicants, such as bacteria, viruses, parasites, or chemical substances, can cause more than 200 diseases. Pathogens that cause diarrheal diseases alone affect over 550 million people, and lead to 230,000 deaths annually [3]. Additionally, in 2017, high sodium intake and low consumption of whole grains, and fruits were responsible for 11 million deaths and 255 million disability-adjusted life years [4].

Sometimes, the health consequences of unhealthy food are due to the way it is prepared. For example, fried foods are often considered unhealthy because they contribute to the global disease burden [5]. A study has shown that frequent consumption of fried foods is linked to an increased risk of various chronic diseases, such as Type 2 diabetes, cancer, cardiovascular disease, autoimmune diseases, and more [6].

Foods are commonly fried using edible oil, which serves as the medium for heating and cooking. Oils are often reused multiple times for frying, which can compromise the nutritional integrity of the fried foods. Unfortunately, the practice of using repeatedly heated oil is widespread in both restaurants and households, with little awareness of its health consequences [7]. This study aims to investigate the quality of edible palm olein oil that has been repeatedly heated.

Palm olein is the liquid fraction of palm oil, obtained through refining, bleaching, and deodorization [8]. It is a rich source of monounsaturated fats, primarily oleic acid (around 35-45%), and also contains significant amounts of palmitic acid (around 36-52%) and linoleic acid (around 10-15%) [9-11]. Palm olein oil has been reported to contain squalene, Vitamin E, carotenoids, and oleic acid [12].

Like other edible oils, palm olein oil is commonly used for frying. However, when used repeatedly, it undergoes thermal oxidation, polymerization, and cyclization [13]. As a result, the chemical constituents of the oil degrade due to exposure to high temperatures, oxygen, and moisture, generating toxic metabolites [14]. Some of these toxic products, such as hydroperoxides and aldehydes formed during repeated heating, are absorbed by the food and enter the systemic circulation after consumption [15]. These hydroperoxides and aldehydes have been shown to pose significant health risks, including an increased risk of diabetes, certain cancers, and cardiovascular diseases [16].

Assessing the quality of palm olein oil is crucial for advancing and disseminating knowledge. A study among residents of Kuala Lumpur regarding the use of repeatedly heated cooking oil revealed that a large proportion of respondents are aware that consuming such oil is harmful to health. However, they are unaware of some specific health complications associated with its use [17].

A similar study among night market food outlet operators in Kuala Lumpur found that most respondents admitted to frequently reusing cooking oil. Although most agreed that using repeatedly heated cooking oil is detrimental to health [18].

Repeated heating of vegetable oil alters its physical and chemical properties, making it harmful for human consumption [19]. This process leads to the formation of free radicals and oxidative stress, ultimately causing damage to consumers at the cellular and molecular levels [20]. Experimental studies have shown that consuming repeatedly heated oils can result in changes in blood chemistry and damage to vital organs [21].

A recent study revealed increased levels of 3-monochloropropanediol (3-MCPD) in soybean and olive oils that have been repeatedly heated [22]. 3-MCPD has been classified as a potential human carcinogen by the International Agency for Research on Cancer [23]. Long-term oral exposure to 3-MCPD is dangerous for various organs, including the heart, liver, immune system, neurological system, and reproductive system. It can also lead to nephropathy, tubular hyperplasia, and adenoma [24]. Consuming repeatedly heated oil may also be linked to a higher recurrence rate of hypertension [25]. These are just a few of the numerous health effects of consuming oil that has been repeatedly fried.

Edible oil is an important and integral component of food. It supplies energy, serves as a carrier for fat-soluble vitamins, and acts as a precursor for the synthesis of steroid hormones and prostaglandins, among other functions [26]. This implies that humans cannot do without it. However, precautions must be taken on how the oil is used. Many studies evaluated the quality of repeatedly heated oils used in deep-frying foods, especially sweet potatoes. However, little is known about the quality of oil repeatedly used for deep-frying fish fries. Therefore, the aim of this study was to investigate the chemical characteristics of non-fried oil, and repeatedly fried oils.

Materials and method

Prior to the analysis, a cross-sectional survey was conducted among 365 respondents to obtain information on the type of oil most frequently used for frying. The study location was Zaria and was selected through a multi-stage sampling technique. Information on the most frequently fried food, duration of frying, and the number of times the oil was repeatedly used for frying was obtained. Data were collected from the respondents through face-to-face interviews using a semi-structured questionnaire.

Palm olein oil was identified as the most frequently used oil and was randomly purchased on the March 12, 2022, from Tudun Wada Market, Zaria Local Government, Kaduna State, Nigeria. Fish was identified as the most frequently fried food. The frying duration ranged from 30 to 60 minutes, with an average of 45 minutes adopted as the standard duration. Independent frying was performed 3 times (n = 3) and 10 times (n = 10). The fish was fried using palm olein oil under the mentioned conditions. The oil-to-fish ratio used in this study was 3:1.

In the oil fried 10 times, approximately 15g of oil and 5g of fish were used initially. Thereafter, a 3:1 ratio of oil to fish was maintained throughout the frying process. For the oil fried three times, about 6g of oil and 2g of fish were used for the first frying. The 3:1 ratio of oil to fish was then maintained throughout the process. The amount of oil remaining after each frying cycle determined the quantity of fish used for subsequent frying.

An average temperature of 182°C was recorded during the frying process. The frying process was repeated every 24 hours using the same oil.

Determination of chemical constituents of non-fried oil, three times, and ten times fried oils

The chemical characteristics of the oils were done using Gas chromatography Mass Spectrophotometer. The analysis was carried out 07-July-2022 in Multi-User Science Research Laboratory, Ahmadu Bello University, Zaria, Nigeria.

Results

Some chemical constituents of non-fried palm olein oil

Table 1 displays the chemical constituents, including the fatty acid profile, of non-fried palm olein oil. Various chemical components identified by Gas Chromatography Mass Spectrometer (GC-MS) were detected in the non-fried palm olein oil. Oleic acid (9-octadecenoic acid) (33.48%) was the primary fatty acid component in the non-fried palm olein oil (Table 1).

Other fatty acids present included 9, 12-octadecadienoic acid (8.16%), commonly known as linoleic acid or omega-6 fatty acid, and hexadecanoic acid (palmitic acid) (7.08%). Table 1 also revealed the derivatives of oleic acid and stearic acid: 8,11-Octadecadienoic Acid, methyl ester (0.68%), and 11-Octadecenoic Acid, methyl ester (2.01%). Additionally, 2-octanoic acid (caprylic acid) and 2-nonynoic acid were present in the composition in minor amounts: 0.74% and 1.71%, respectively. The fresh palm olein oil also contains trans-fat, methyl 9-cis., 11-trans.,13-trans.-octadecatrienoate (2.69%) (Table 1).Cyclopentaneundecanoic acid (3.38%), 9-Oxabicyclo[6.1.0]nonane (13.56%), and 2-(2-Methyl-acryloyl)-cyclohexanone (3.67%) were identified in the fresh palm olein oil. Benzene-containing compounds such as benzene, octyl- (2.89%) and benzene, nonyl- (3.49%) were also found. Other compounds present include glycerol 1-palmitate (0.57%), oxalic acid, decyl 1-menthyl ester (0.66%), (3E,7E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (5.64%), 12-Methyl-E,E-2,13-octadecadien-1-oldl-.alpha-Tocopherol (4.75%), and 1,1-bis(difluoromethyl)ethyl isocyanate (0.48%). Additionally, eicosanoic acid (3.78%) was detected (Table 1).

Table 1. Fatty acid and some chemical constituents of non-fried palm olein oil

Chemical constituents of fresh oil	Composition (%)
2- Octanoic acid	0.74
2-Nonynoic acid	1.71
Benzene, octyl-	2.89
Oxalic acid, decyl 1-menthyl ester	0.66
Benzene, nonyl-	3.49
Hexadecanoic acid	7.08
8,11-Octadecadienoic acid, methyl ester	0.68
11-Octadecenoic acid, methyl ester	2.01
9,12-Octadecadienoic acid	8.16
Octadecanoic acid	0.59
Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate	2.69
Glycerol 1-palmitate	0.57
9-Oxabicyclo[6.1.0]nonane	13.56
(3E,7E)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene	5.64
12-Methyl-E,E-2,13-octadecadien-1-ol dl-.alpha.-Tocopherol	4.75
2-(2-Methyl-acryloyl)-cyclohexanone	3.67
1,1-Bis(difluoromethyl)ethyl isocyanate	0.48
9-Octadecenoic acid (Oleic Acid)	33.48
Cyclopentaneundecanoic acid	3.38
Eicosanoic acid	3.78

Fatty acid and some chemical constituents of three times fried palm olein oil

The percentage composition of oleic acid in fresh palm olein oil was reduced to 17.08% in palm olein oil that had been fried three times (Table 2). Additionally, several cyclic compounds were identified in the three times fried oil, including 2,4,6-cycloheptatrien-1-one, 3,5-bis-trimethylsilyl- (17.57%), cyclotrisiloxane, hexamethyl- (14.26%), cyclopentadecane (0.56%), and cyclododecanol, 1-ethenyl- (0.94%) (Table 2).

Other compounds such as hexacosyl propyl ether (0.40%), 17-pentatriacontene (1.35%), dihydro citronellyl angelate (0.89%), hexadecane, 2,6,10,14-tetramethyl- (2.51%), decane (1.45%), tetracontane, 3,5,24-trimethyl- (1.31%), 1-decanol, 2-octyl- (0.66%), lauroyl peroxide (1.54%), 2-methyl-E-7-octadecene (7.50%), heptane, 2,6-dimethyl- (0.33%), octadecane, 1-(ethenyloxy)- (10.37%), squalene (0.81%), tricosanoic acid, isobutyl ester (1.16%), 2-ethylacridine (13.97%), arsenous acid, tris(trimethylsilyl) ester (0.64%), and cholesterol (2.76%) were reported in the three-time fried palm olein oil (Table 2). Results in Table 2 also showed that hexadecanoic acid and cis-vaccenic acid were present in compositions of 0.54% and 1.39%, respectively.

Fatty acid and some chemical constituents of 10 times fried palm olein oil

The results presented in Table 3 reveal that oleic acid, found in oil fried 10 times, had a reduced percentage composition (14.15%). Additionally, hexadecanoic acid (2.49%) and pentadecanoic acid (0.77%) were detected. Furthermore, more polycyclic compounds such as 2,4,6-Cycloheptatrien-1-one (19.15%), Cyclobutyl-heptadecyl ester (2.45%), and Oxacyclotetradecane-2,11-dione, 13-methyl (1.37%) were generated (Table 3).

Benzene-containing compounds 1,4-bis-(trimethylsilyl)-benzene (15.54%) and 1,2-benzisothiazol-3-amine (23.08%) were also revealed in edible oils fried 10 times. Additionally, as shown in Table 3, the oil fried 10 times contains, to a lesser extent, other compounds: 1-octadecene (0.12%), carbonic acid (0.30%), 1,4-octadiene (0.10%), and 1-nonadecene (0.19%) (Table 3).

Table 2. Fatty acid and some chemical constituents of three times fried oil

Chemical constituents	Percentage composition (%)
Hexacosyl propyl ether	0.40
17-Pentatriacontene	1.35
Dihydro citronellyl angelate	0.89
Cyclopentadecane	0.56
Hexadecane, 2,6,10,14-tetramethyl-	2.51
Cyclododecanol, 1-ethenyl-	0.94
Decane	1.45
Tetracontane, 3,5,24-trimethyl-	1.31
n-Hexadecanoic acid	0.54
cis-Vaccenic acid	1.39
Oleic Acid	17.08
1-Decanol, 2-octyl-	0.65
Lauroyl peroxide	1.54
2-Methyl-E-7-octadecene	7.50
Heptane, 2,6-dimethyl-	0.33
Octadecane, 1-(ethenyloxy)-	10.37
S qualene	0.81
Tricosanoic acid, isobutyl ester	1.16
Cholesterol	2.76
2-Ethylacridine	13.97
2,4,6-cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	17.57
Arsenous acid, tris(trimethylsilyl) ester	0.64
Cyclotrisiloxane, hexamethyl-	14.26

Table 3. Fatty acid and some chemical constituents of 10 times fried oil

Chemical constituents	Percentage composition (%)
1-Octadecene	0.12
Carbonic acid	0.30
1,4 – Octadiene	0.10
1 – nonadecene	0.19
n-hexadecanoic acid	2.49
Pentadecanoic acid	0.77
Cyclobutyl-heptadecyl ester	2.45
Oxacyclotetradecane-2,11-dione,13-methyl	1.37
Trans-13-octadecenoic acid	1.63
Oleic acid	14.15
Supraene	0.26
Cholesterol	3.40
2-ethylacridine	11.69
Propanamide	3.31
2,4,6-Cycloheptatrien-1-one	19.15
1,4-Bis-(trimethylsilyl)-benzene	15.54
1,2-Benzisothiazol-3-amine	23.08

Discussion

The gas chromatography-mass spectrometry (GC-MS) analysis of fresh palm olein oil revealed that oleic acid (33.48%) was the major fatty acid constituent. A study reported that palm olein contains higher levels of total oleic acid (39–45%) compared to palm oil. Further processing can produce palm super olein (iodine value > 60) and palm top olein (iodine value, 70–72) [27]. The percentage composition (33.48%) of oleic acid revealed in this study is comparable to that reported in another study [28]. Higher plasma levels of oleic acid appear to be a risk factor for cardiovascular disease (CVD) events [29], while another study suggested that higher intake of oleic acid could reduce the incidence of CVD [30]. The health benefits of oleic acid have been demonstrated in studies showing that it helps reduce cardiovascular risks in populations consuming a Mediterranean diet [31] and provides protection against cardiovascular insulin resistance in the atherosclerotic process [32].

The GC-MS analysis also revealed the presence of 9-Oxabicyclo[6.1.0]nonane (13.56%). 9-Oxabicyclo[6.1.0]non-4-ene, also known as 9-Oxabicyclononane, is a bicyclic hydrocarbon with a single oxygen atom located at the 9th carbon position. The implications of 9-Oxabicyclononane in relation to cardiovascular disease risk are currently limited.

Linoleic acid (9,12-Octadecadienoic) was found in fresh palm olein oil in a small amount (8.16%). This is similar to the composition (8.00%) of linoleic acid found in a study that analyzed the fatty acid composition of various vegetable oils from Indonesia [33]. However, the percentage composition of linoleic acid in their study was from unrefined palm oil.

The consumption of linoleic acid is generally promoted as beneficial for human health, particularly for cardiovascular health, but there is controversy regarding the optimal intake for health benefits [34]. Therefore, consuming a significant amount of linoleic acid may pose a health risk, given its half-life is approximately two years [34].

The trans-fatty acid content observed in this study was higher than the composition (0.39–0.84%) reported in palm olein oil by Hishamuddin et al [35]. Additionally, levels of nonyl-benzene (3.49%) and octyl-benzene (2.89%) were detected in the palm olein oil. These benzene derivatives may result from the use of food-grade hexane for extracting edible oil by production companies, as mentioned by Joshi et al [36]. Benzene, in general, has been implicated in the etiology of cardiovascular disease (CVD) [37].

Traces of cyclopentaneundecanoic acid (3.38%) and eicosanoic acid (3.78%) were found in the palm olein oil. Cyclopentaneundecanoic acid, also known as hydnocarpic acid, is a monounsaturated long-chain fatty acid consisting of undecanoic acid with a cyclopent-2-enyl group at the 11-position. Unlike the monocyclic aromatic hydrocarbons such as nonyl-benzene, octyl-benzene, and cyclopentaneundecanoic acid identified in this study, Liu et al. [38] discovered that certain cooking oils used in China contained polycyclic aromatic hydrocarbons.

An observation in this study revealed that frying fresh palm olein oil three times resulted in a nearly 50% decrease in oleic acid content. This decrease in oleic acid may be attributed to its degradation. A study found that the degradation of oleic acid in repeatedly fried oil is primarily caused by thermo-oxidative and hydrolytic reactions driven by high temperatures, the presence of oxygen, and moisture from the food [39]. Thermo-oxidation involves processes such as hydrolysis, oxidation, polymerization, and isomerization. The decrease in oleic acid content could be due to its unsaturated nature, as the amount of unsaturated fatty acid can also influence the degradation of frying oils [40].

This study suggests that water from the fish breaks down triglycerides into free fatty acids (FFAs), while oxygen from the environment reacts with fatty acids in the palm olein oil. The fatty acids are then linked together through polymerisation to form larger polar compounds [41]. Furthermore, the cis-fatty acids in the oil matrix can be converted to trans-fatty acids [42].

As mentioned earlier, fresh palm olein oil has an oleic acid content of 33.48%, whereas palm olein oil fried three times has an oleic acid content of 17.08%. The observed decrease is an indication that repeated frying of the oil caused changes in the fatty acid composition, which is consistent with similar observations in a study [43].

It was observed that aromatic compounds increased in oil that had been fried three times, as indicated by the compositions of 17.57% and 14.26% for 2,4,6-cycloheptatrien-1-one, 3,5-bis-trimethylsilyl, and Cyclotrisiloxane, hexamethyl, respectively. The formation of 2,4,6-cycloheptatrien-1-one, 3,5-bis-trimethylsilyl, and cyclotrisiloxane, hexamethyl may not result from repeated frying of the oil. It is possible that both compounds were introduced during analysis, likely as artefacts of the sample preparation process, specifically the silylation derivatization, rather than being produced in the oil during repeated frying. Silylation derivatization is a chemical process that replaces active hydrogen atoms (like -OH, -NH, -SH, -COOH) in polar molecules with a less polar, more volatile silyl group (like trimethylsilyl or TMS), making them suitable for gas chromatography (GC) or mass spectrometry (MS) analysis by improving their volatility, thermal stability, and chromatographic separation [44].

Studies have also reported higher concentrations of aromatic compounds in oil that has been repeatedly fried [45, 46]. Although research has shown that aromatic compounds generated in repeatedly fried oil may pose health challenges such as cardiovascular issues, cancer, and neurodegenerative diseases [47]; information on the health implications of 2,4,6-cycloheptatrien-1-one and Cyclotrisiloxane, hexamethyl is limited.

In the 10th round of frying, the percentage composition of oleic acid decreased to 14.15%, while the concentration of aromatic compounds increased. A study by Sadawarte and Annapure [48] discovered that repeated deep frying degrades the quality of frying oil over time. Their research showed a decrease in oleic acid composition from 42.4% to 38.9% after 16 cycles of frying. In contrast, in our study, the percentage of oleic acid dropped from 33.48% to 14.15% after 10 cycles, indicating a greater reduction than in their study. This difference could be attributed to the temperature difference, as this study used 182°C compared to 160 ± 5 °C in their study. The percentage compositions of 1,2-Benzisothiazol-3-amine, 2,4,6-Cycloheptatrien-1-one, and 1,4-Bis-(trimethylsilyl)-benzene were found to be 23.08%, 19.15%, and 15.54% in the 10 times (nine times repeatedly) fried oil. The increased concentration of these compounds in repeatedly fried oils makes them unhealthy for consumption.

The cholesterol found in oils that are repeatedly used for frying may result from the transfer of it from the fried fish into the oil, rather than from the repeated use of the oils themselves. This is supported by the fact that plant-based oils do not naturally contain cholesterol. If cholesterol is repeatedly heated over time, it can lead to the formation of toxic metabolites, which may have negative effects on health.

Repeatedly frying oils can also lead to the formation of harmful cholesterol oxidation products known as oxysterols. Research shows that using an air fryer can increase the levels of cholesterol oxidation products (COPs) in fish [49] and other animal-based foods [50].

Conclusion

Edible oils are essential ingredients for frying, but they are often used repeatedly. Therefore, this study investigated the chemical changes in edible oil used repeatedly for frying fish. The results showed that the fatty acid profiles of the oil decreased after three and ten uses. Mono-cyclic aromatic hydrocarbons were also revealed in the repeatedly fried oils. These compounds may be associated with various human diseases such as cancer, dementia, and especially cardiovascular diseases. Therefore, nutrition education highlighting the dangers of consuming repeatedly fried oil is crucial.

Supplementary material

The supplementary material of this reseach is available at: https://file.luminescience.cn/FNDS-499%20Supplementary%20material.pdf.

Authors' contribution

Conceptualization – SOA and IA; Methodology – SOA, IA and RAM; Formal Analysis- IA, SOA, HA and ASA; Data Curation – OSO, FI and IA; Original Draft Preparation – SOA, IA, HA, OSO, RAM, FI, and ASA; Review & Editing – SOA, IA and RAM; Visualization- HA, FI, and ASA; Supervision- RAM and IA; Project Administration- SOA, IA, HA, OSO, RAM, FI, and ASA.

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Conflict of interest

The authors declare no conflict of interest.

Declarations

Not applicable.

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