Toxicological Potentials Of Repeated Frying On Antioxidant Status Of Vegetable Oils

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Abstract

The process of repeated frying of dietary vegetable oils plays a significant role in the generation of free radicals, which could be determined on the level of performance, lipid peroxidation and antioxidant defense system respectively. This study was conducted to investigate the possible toxicological effects of repeated frying on the levels of β–carotene, α-tocopherol and malondialydehyde of some commercially edible vegetable oils. Ten different samples of vegetable oils were thermally stressed through repeated frying for three consecutive days. The results indicated that the amount of malondialdehyde formation was highest in the rancid palm oil sample (from 0.166 mmol/ml in day 0 to 0.212 mmol/ml in day 3), while fresh palm oil sample had the lowest amount of malondialdehyde formation (from 0.025 mmol/ml in day 0 to 0.041 mmol/ml in day 3). The refined deodorized palm olein, groundnut oil, congealed and locally made vegetable oil samples also showed a significant increase ($P < 0.05$) in the concentration of malondialdehyde with repeated frying of oils. The significant increase ($P < 0.05$) in the levels of MDA was marked by a significant decrease ($P < 0.05$) in their β–carotene and α-tocopherol levels during the course of repeated frying of the oils.

1. Introduction

In today's society with all its modern trappings, repeated frying with vegetable oils is a widely used procedure for meal preparation [1,*2*]. During deep fat frying, oil is heated at a temperature above 150^0C for a long time in the presence of air. Under these conditions, both oxidation and thermally breakdown of the oil may occur. These productions have toxic effects and may cause different adverse effects [3,4]. Vegetable oils especially palm oil regardless of their sources, exhibit good frying performance, which contributes to their widespread use in deep-frying applications [*5, 6*]. Due to the rising demand and increase in fat intake, palm oil is the major oil in the world's oil and fat market, and palm oil is projected to remain the most influential fat source. The refined, bleached deodorized palm olein, which is fractionated from palm oil, is commonly used as cooking oil. It offers better resistance to oxidation of high temperature during frying as well as natural antioxidants from the vitamin E group, the tocotrienols, etc [*7, 8*].

The use of fat or oil for frying still remains one of the most popular methods for the preparation of foods. When frying oil is heated, hydroperoxides and aldehydes are formed [*9*]. These oils are susceptible to oxidative changes. The necessity of using a good quality frying medium becomes obvious when one considered that some of the fats are absorbed by every piece of food fried [10]. The overuse of deep frying of vegetable oil causes adverse effects on flavour, stability, colour and texture of fried products and may be harmful to human health. The degradative product formed during repeated frying includes both volatile and non-volatile compounds, although most of the volatile compounds are lost during the frying process [10]. These compounds are of concern because they accumulate in the frying oil,

promote further degradation, absorbed by the fried food, enter the diet and affect public health [11].

The main economic factor considered in fried food products is the cost of vegetable oils because they are the major ingredients in these products. Therefore, very often the oil is repeatedly used to minimize the expense of food preparation. During the reheating process, the oil undergoes various physical reactions such as formation of foam, increase in viscosity, darkening of colour and deterioration of flavour. These changes may affect the organoleptic qualities such as the odour and taste, and the nutritional value of the fried foods [6, 12]. Furthermore, repeated frying causes chemical reactions such as hydrolysis, oxidation and polymerization that may alter the chemical structure of triacylglycerol molecules, of which Poly Unsaturated Fatty Acids (PUFA) molecules are affected the most [13]. Thermally oxidized oils such as those produced by repeated frying, contain a complex mixture of products, such as oxidized monomers, dimmers and polymers. These products have been reported to be the substances mainly responsible for changes in the physicochemical properties of fats [14].

Free radicals generated during repeated frying process could damage lipids by initiating lipid peroxidation. Malondialydehyde, one of the major secondary oxidation end products of peroxidised PUFA, has been shown to be of biological significance[15,16]. Although, α-tocopherol and β-carotene are major non-enzymatic antioxidants, the ingestion of oxidized oils also reduces their levels in tissues [17, 18, 19] and increases the amount of thiobarbituric acid – reactive substances (TBARS), suggesting an elevation in lipid peroxidation and cellular fragility [17, 20]. In view of the potentially hazardous effect of ingested heated oils on human health, this present study was planned to ascertain the toxicological effects of repeated frying of some commercially edible vegetable oils on the respective levels of malondialdehyde, α-tocopherol and β-carotene. This gives us the insinuation on how to compare and correlate the results obtained from the aforementioned analysis with those from the same vegetable oils used for the repeated frying of fish for three consecutive days.

2.0. Materials and Methods

2.1. Source of Vegetable Oils

Nine samples of the cooking oils were purchased from Igbudu market in Warri-South Local Government Area of Delta State, Nigeria. The tenth sample (fresh oil) was locally prepared by us. In the local preparation of the oil; the purchased palm fruits were kept for about a week for adequate putrefaction of the nuts thereafter, subjected to boiling for 30-60minutes. The palm fruits were gently pulverized using the native mortar and pestle, where the juices were extracted and allowed to stand on exposure to sun for 7-12 days. The locally made oil was finally obtained by simple decantation from the extract. However, the palm fruits which were used for the extraction of the oil were purchased from Ogharefe market in Ethiope-West Local Government Area of Delta State, Nigeria.

2.2. Fish Preparation, frying protocol and Oil sampling

The various brands of vegetable oils were used to fry fishes as follows; the fishes (Mackerel) were washed with distilled water and cut into pieces; 10g of the fishes were fried with 1000 ml of the vegetable oils separately in a deep fryer (Eurosonic, China) at 150^{o}C for 7minutes during three consecutive days. The fryer was left uncovered during the frying period. The fryer was turned off at the end of the frying experiment each day and the oil was allowed to cool to 60^{o}C.The Oil in the fryer was then filtered to remove debris using separate filters. Thereafter, the oil samples including the fresh one (zero time) were stored in brown bottles for subsequent chemical analyses.

2.3. Biochemical Assays

β-carotene and α-tocopherol levels in the used oil samples were estimated by using the method developed by Jakutowicz *et al* (21). For β-carotene assay, 1.0ml of supernatant from sample was pipetted into a test tube and then 0.5ml of absolute ethanol was added, followed by 4.0ml petroleum ether. The solution was read at 450nm. The amount of β-carotene present was calculated using molar extinction coefficient of $1.38\times10^{5}M^{-1}cm^{-1}$. Results were presented as means±S.D. of triplicate determination.

α-tocopherol levels in oil samples were determined as follows; 1.0ml of supernatant was measured in a test tube and 0.5ml of absolute ethanol added. 4ml of petroleum ether was subsequently added into the test tube followed by 2ml of 4, 7-diphenyl-1, 10-phenathraline and absorbance read at 534nm. The amount of α-tocopherol present was calculated using molar extinction coefficient of $1.36 \times 10^{5}M^{-1}cm^{-1}$. Results were presented as means±S.D. of triplicate determination.

Lipid peroxidation in the used oil samples, as determined by the MDA levels, was measured

using the modified method developed by Maduka and Okoye (22).

In the MDA assay method, 1ml of the oil sample(s) was added to 3ml of buffer solution in test tube of 40% TCA (0.25 ml) and 0.125 ml of 5NHCl was then added and after mixing, 0.25 ml of 2% 2 – Na-thiobarbituric acid was added promptly. The tubes were stoppered with cotton wool and placed in boiling water for ten minutes, cooked and centrifuged at 2500rpm for ten minutes and the absorbance of the colour change was read at 532nm. The MDA formed was calculated using a molar extinction coefficient of 1.56×10^5 $m^{-1}cm^{-1}$ and 46 as constant factor of lipid peroxidation was used to multiply the resulting value.

2.4. Statistical Analysis

The results for the β-carotene, α-tocopherol and lipid peroxitation (MDA) levels were expressed as means ± standard deviation(SD).Data were subjected to one-way analysis of variance (ANOVA) with least significant difference and Duncan post-hoc test for differences between pairs of means when applicable. A value of $P < 0.05$ was considered significant. All analysis was conducted using statistical product and service solutions (SPSS) software package (version 20.0).

3. Results

Ten varieties of edible vegetable oils subjected to repeated frying for three consecutive days had been analyzed. We observed a significant effect ($P < 0.05$) on the concentration of MDA in the various vegetable oil samples, as it increased progressively during the repeated frying process (Table 1.0).We also observed a significant reduction ($P < 0.05$) in the β-carotene and α-tocopherol levels at different rates during the frying of the oils for three consecutive days (table 2.0 and table 3.0).

Table 1.0: The Effect of repeating frying on the concentration of Malondialdehyde (mmol/ml x 10^{-3}) in various vegetable oil samples

SAMPLES	DAY 0	DAY 1	DAY 2	DAY 3
FPO	25 ± 3	31 ± 1	35 ± 2	41 ± 1
EPO	52 ± 4	59 ± 1	63 ± 1	66 ± 2
NPO	38 ± 2	39 ± 4	41 ± 2	44 ± 2
RPO	166 ± 1	183 ±18	205 ± 2	212 ± 4
RDPO I	101 ± 1	104 ± 2	108 ± 1	111 ± 2
RDPO II	34 ± 2	38 ± 1	79 ± 19	119 ±17
RDPO III	104 ± 9	100 ± 1	108 ± 2	110 ± 1
CVO	97 ± 9	104 ± 8	110 ± 1	109 ± 1
LMO	87 ± 3	90 ± 1	95 ± 2	96 ± 1
GO	105 ± 2	114 ± 1	125 ± 2	462 ± 1

Values represent means ± SD from three replicates, results were considered significant (P<0.05).

FPO = Fresh palm oil, EPO = Ekpophrephre palm oil, NPO = Native Palm Oil, RPO = Rancid Palm Oil, RDPO I = Refined Deodorized Palm Olein I, RDPO II = Refined Deodorized Palm Olein II, RDPO III = Refined Deodorized Palm Olein III, CVO = Congealed Vegetable Oil, LMO = Locally Made Oil, GO = Groundnut Oil

Table 2.0: The Effect of repeating frying on the levels of Beta-carotene (mmol/ml x 10^{-3}) in vegetable oil samples

SAMPLES/ DAYS	DAY O	DAY 1	DAY 2	DAY 3
FPO	11.6±0.1	10.8 ±0.1	10.7 ± 0.3	9.9 ±0.1
EPO	7.4 ± 0.6	7.2 ± 0.2	7.0 ±0.1	6.9 ± 0.1
NPO	7.5 ±0.2	7.4 ± 0.1	7.3 ±0.1	7.2 ± 0.4
RPO	1.0 ±0. 4	0.9 ± 0.1	0.7 ±0.9	0.4 ± 0.3
RDPOI	4.6 ±0. 5	4.4 ± 0.1	4.2 ±0.3	3.9 ± 0.2
RDPOII	2.3 ± 0.1	2.1 ± 0.1	2.0 ±0.2	1.8 ± 0.1
RDPOIII	1.3 ± 0.6	1.0 ± 0.2	0.9 ±0.1	0.7 ± 0.1
CVO	1.5 ± 0.1	0.7 ± 0.2	0.5 ±0.2	0.4 ± 0.1
LMO	2.3 ± 0.2	2.1 ± 0.4	2.0 ±0.9	1.9 ± 0.1
GO	2.0 ± 0.2	1.7 ± 0.7	1.5 ±0.3	0.3 ± 0.5

** Values represent means ± SD from three replicates, results were considered significant (P<0.05),*

Table 3.0: The Effect of repeating frying on the levels of Alpha-tocopherol (mmol/ml x 10^{-3}) in vegetable oil samples for three days.

SAMPLES/ DAYS	DAY O	DAY 1	DAY 2	DAY 3
FPO	11.2±0.3	9.7 ±0.1	8.9 ± 0.2	8.6 ± 0.1
EPO	1.5 ± 0.1	0.9 ±0.4	0.6 ± 0.1	0.5 ± 0.1
NPO	9.1 ± 0.7	8.9 ±0.5	8.7 ±0.1	8.6 ± 0.2
RPO	1.5 ± 0.7	0.9 ±0.5	0.6 ± 0.2	0.5 ± 0.1
RDPO I	4.8 ± 0.1	4.6 ±0.4	0.43±0.1	4.2 ± 0.1
RDPO II	6.1 ± 0.2	4.8 ±0.1	4.7 ±0.5	4.3 ± 0.3
RDPO III	2.0 ± 0.1	1.8 ±0.1	1.6 ±0.2	1.3 ± 0.1
CVO	5.5 ± 0.1	5.3 ±0.1	5.1 ±0.3	5.0 ± 0.1
LMO	2.0 ± 0.1	1.9 ±1.1	1.7 ±0.2	1.6 ± 0.1
GO	3.0 ± 0.1	0.8 ±0.4	0.6 ±0.1	0.2 ± 0.3

** Values represent means ± SD from three replicates, results were considered significant (P<0.05),*

4. Discussion

In recent times, frying remains one of the most popular methods for food preparation. However, the engagement of this technique in food preparation has been abused through its repeated use. It should be noted, therefore, that the common practice of repeatedly using oil for frying may generate free radicals that are detrimental to human health [15]. For this reason, this study was conducted to ascertain the effects of repeated frying of vegetable oil on factors related to oxidative stress, particularly the formation of lipid peroxidation product , malondialdehyde and some non-enzymatic antioxidants such as β-carotene and α-tocopherol levels in the oil samples respectively. When frying oil is heated at high temperature, toxic products such as hydroperoxides and aldehydes, are formed, absorbed by the food, and subsequently absorbed into the gastrointestinal system and introduced into system circulation after consumption [9, 23].

The results obtained from this study showed a marked significant ($P < 0.05$) increase in MDA levels with repeated frying of the oil samples and a significant ($P < 0.05$) decrease in the antioxidant levels of the oil samples. The loss in the protective effect of the oil could be attributed to the destruction of the heat-labile vitamins such as vitamin E [25]. The potential hazardous effect of heated oils on health led to another study carried out by *Siti et al* [24]. Their work was planned to determine the effects of thermally oxidized palm oil taken together with 2% cholesterol diet on the factors related to atherosclerosis in female rats made oestrogen – deficient by ovariectomy. This gave information on the effects of re-heated palm oil on post-menopausal women in particular and the population in general. The results from their study showed that repeatedly heated palm oil appears to increase lipid peroxidation and cholesterol in a post-menopausal rat model. Also, Owu *et al*, [25] reported that consumption of oxidized oil caused liver dysfunction. The results obtained in this study showed consonance with the aforesaid reports.

In this present study, the results indicate that palm oil samples with the exception of rancid palm oil were less susceptible to oxidation than the other vegetable oil samples. This could be as a result of the highest provitamin A activities in palm oil relative to other vegetable oils [26,27]. The groundnut oil sample on the other hand displayed higher levels of MDA concentration with repeated frying compared to those of other vegetable oil samples, except rancid palm oil sample. This was marked by a decrease in the antioxidant levels of the groundnut oil. The increase in the MDA levels in comparison to that of palm oil samples could be attributed to its higher composition of polyunsaturated fatty acids than palm oils [26]. Polyunsaturated fatty acids (PUFA) of these oils readily undergoes oxidation resulting in the formation of peroxides, aldehydes, ketones, aldehydroesters and ozonids [27, 28]. The losses in the α-tocopherol levels of the groundnut oil agrees with the report of *holownia et al* [29], which showed that tocopherol losses were observed in peanut oil used for the repeated frying of both marinated and non-marinated chickens.

Refined deodorized palm olein sample also showed significant ($P < 0.05$) increase in their MDA levels with an attendant decrease in their α-tocopherol and β-carotene levels respectively. These findings correlate with the report of *Schroeder et al* [30] in which the fatty acid profiles and oxidative stability of yellow or red palm olein samples during the repeated frying of potato chips was studied. The results indicated that the antioxidant level of the palm olein samples decreased with repeated frying of potato chips. Congealed and locally made oils exhibited a different pattern of MDA increment and a marked reduction in the β-carotene and α-tocopherol levels of the oil sample respectively.

The increase in the levels of MDA in the vegetable oil samples with repeated frying could also be attributed to the fish fried in it. Fish oils contain omega – 3 fatty acids, especially Eicosapentaenoic acid and Docosahexaenoic acid, which are polyunsaturated. These polyunsaturated oils are susceptible to oxidation [31].

5. Conclusion

In conclusion, the repeated use of fried vegetable oils for cooking should be avoided regardless of the economic disposition, especially by post-menopausal women. Among the varieties of vegetable oils, palm oil with high antioxidant activity and less susceptibility to oxidation should be preferred for cooking and should be used with utmost caution so as to avoid the deleterious effects of fried oils to human health in general.

6. References

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