

Review Article

Effects of Repeated Heating of Cooking Oils on Antioxidant Content and Endothelial Function

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Reusing cooking oil in food preparation, especially during deep-frying, is a common practice to save costs. Repeated heating of the oil accelerates oxidative degradation of lipids, forming hazardous reactive oxygen species and depleting the natural antioxidant contents of the cooking oil. Long-term ingestion of foods prepared using reheated oil could severely compromise one's antioxidant defense network, leading to pathologies such as hypertension, diabetes and vascular inflammation. The detrimental effects of reheated oil consumption extend beyond mere oxidative assault to cellular antioxidant shield. In this review, we have examined the experimental and clinical effects related to the intake of reheated oil on antioxidant contents, membrane lipid peroxidation and endothelial function. Understanding the mechanisms underlying the pathology associated with intake of repeatedly heated oil will help to set a reference for assessing the safety of cooking oil. Finally, considering the potential hazard of repeatedly heating oil, this article aims to further increase awareness of the general public regarding the health risks associated with these oils.

Keywords: Antioxidant; Endothelial dysfunction; Heating; Lipid peroxidation; Oxidative stress; Vegetable oils**Introduction**

According to the United States Department of Agriculture, 168.85 million metric tons of vegetable oils are estimated to be produced globally at the end of 2013-2014 season [1]. World vegetable oil production has increased over the past decades, especially production of palm oil, soybean oil, rapeseed oil (canola) and sunflower oil (Table 1). Vegetable oils are regarded as the healthier choice relative to animal fats in view of their unsaturated fatty acid and cholesterol-free contents. In this fast-paced society, frying remains as one of the popular methods in food preparation. Consumption of ready-made deep-fried food is high, especially in developing countries. Highly oxidized fatty acids are consumed through intake of these fried foods. Edible vegetable oil is the major ingredient in these fried food products. Therefore, the cost of the oil becomes the most important factor to be considered in terms of economy. As a result, vegetable oil is often to be repeatedly heated to ensure cost effectiveness. The oil is thus reused until it is discarded and replaced with fresh oil.

When frying oil is heated at high temperatures, hydroperoxides and aldehydes are formed. These toxic products are absorbed by the food, and eventually into the gastrointestinal tract and thereafter enter the systemic circulation after ingestion [2]. We recently reported that intake of repeatedly heated palm and soybean oils significantly increased the blood pressure in experimental animals [3,4]. In addition, Soriguer et al. [5] reported that consumption of repeatedly heated frying oils is associated with increased risk of hypertension. The practice of reusing frying oil leads to detrimental health risks such as histological abnormalities [6-9] and alterations in genetic material [10-12]. Free radicals generated during the frying process could damage membrane lipids through lipid peroxidation, subsequently leading to oxidative stress. This review examines the current

literature on the harmful effects of repeatedly heated vegetable oils on antioxidant activity, lipid peroxidation and endothelial function.

Heating Process of Vegetable Oil

During the frying process, cooking oil is exposed to an extremely high temperature in the presence of air and moisture. Under such conditions, a complex series of chemical reactions takes place, resulting in loss of both quality and nutritional values of the cooking oil. Repeatedly heating the cooking oils initiates a series of chemical reactions, modifying the fat constituents of cooking oil through oxidation, hydrolysis, polymerization, and isomerization, eventually resulting in lipid peroxidation [13]. Lipid peroxidation generates a wide spectrum of volatile or non-volatile components, including free fatty acids, alcohols, aldehydes, ketones, hydrocarbons, *trans* isomers, cyclic and epoxy compounds [14,15]. As a result, when the same cooking oil is reused excessively, the chemical reactions enhance foaming, darkening of oil color, increased viscosity, and off-flavor. Hence, repeated heating of the oil can lead to degradation of the cooking oil, both chemically and physically.

Although the chemical reactions provoked by thermal treatment are complex, they interact with and affect each other. Exposure to oxygen at high temperatures leads to oxidation of triacylglycerides, which generates hydroperoxides. Hydroperoxides are unstable intermediates and rapidly break down into reactive free radicals to initiate autoxidation, generally through a three-phase process (initiation, propagation and termination). Autoxidation is therefore suggested to be a principal mechanism of lipid peroxidation. The extreme heat during frying is the main initiator for autoxidation, in addition to other factors such as photonic agents, ionizing radiation, free radicals and chemical impacts. The initiation phase involves homolytic cleavage of hydrogen bonds, particularly those in the

Table 1: World consumption, fatty acid composition and CVD risk factor of major vegetable oils.

Vegetable oil	World consumption (million metric tons) ¹	Fatty acid (g/100g) ²			Study	Design	Key finding
		SFA	MUFA	PUFA			
Palm oil	56.02	49.30	37.00	9.30	Ladeia et al. [62]	Quasi-experiment	A mild, triacylglycerol-reducing effect in young and healthy subjects
Soybean oil	44.17	15.65	22.78	57.74	Hassan and Abdel-Wahhab [63]	Experimental	Restoration of lipid profile, cardiac biomarkers, inflammatory and redox status, suggesting protection against cardiovascular disorders associated with estrogen deficiency
Rapeseed oil	24.06	7.37	63.28	28.14	Gillingham et al. [64]	Single-blind, randomized, crossover, controlled	Serum total cholesterol and LDL cholesterol are lowered compared to Western diet
Sunflower seed oil	14.07	10.10	45.40	40.10	Binkoski et al. [65]	Double-blind, randomized, crossover, controlled	Total and LDL cholesterol levels are reduced compared to average American diet
Peanut oil	5.56	16.90	46.20	32.00	Stephens et al. [66]	Experimental	Aortic total cholesterol and cholesteryl ester are reduced, demonstrating an anti-atherosclerotic property
Coconut oil	3.82	86.50	5.80	1.800	Mendis et al. [67]	Randomized, controlled	Replacement or reducing the oil intake is associated with the decrease in mean cholesterol levels
Olive oil	3.05	13.81	72.96	10.52	Buil-Cosiales et al. [68]	Retrospective	An inverse association between oil consumption and carotid intima-media thickness, suggesting an anti-atherosclerotic effect

Abbreviations are: CVD: Cardiovascular disease; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; LDL: low-density lipoprotein

¹United States Department of Agriculture. 2013. Oilseeds: world markets and trade.

²United States Department of Agriculture. 2013. National Nutrient Database for Standard Reference, Release 26.

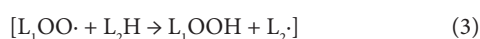
α -position relative to the double bond of the fatty acid chain, to form alkyl radicals ($L_1\cdot$; reaction 1).



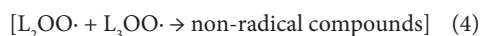
$L_1\cdot$ radicals are highly unstable intermediates. They stabilize themselves by reacting with oxygen to generate peroxyradicals ($L_1OO\cdot$; reaction 2).



The resulting peroxyradical then abstracts a hydrogen from other unsaturated fatty acid (L_2H) to form a hydroperoxide (L_1OOH) and another alkyl radical ($L_2\cdot$; reaction 3), thus replenishing the reaction (1). This phase is called propagation. It propagates sustainably at a high rate.



The propagation phases continue until a maximum concentration of hydroperoxide is reached, at which time point the collision between the individual moieties becomes more frequent. This stage marks the onset of the termination phase, in which the double bond adjacent to the hydroperoxyl group is broken down to yield hydrocarbons, aldehydes, alcohols and ketones (reaction 4).



Hydrolysis, another key pathway of lipid peroxidation, is initiated by water vapor found in food and the atmosphere. Activated water molecules break down the esterified bonds of triacylglycerides to generate glycerol, free fatty acids, monoacylglycerides and diacylglycerides. The breakdown products in turn accelerate the hydrolysis rate. At the same time, high temperatures induce polymerization of the hydrolysis products to form high-molecular-weight cyclic fatty acid monomers, dimers or oligomers, which subsequently speeds up the hydrolytic reaction.

Effect of reheated vegetable oils on antioxidant activity

Excessive generation of reactive oxygen species (ROS), coupled with a reduced availability of antioxidants, predisposes the cells to

a state of oxidative stress. ROS are highly reactive and unstable in nature. Antioxidants present in oil inhibit oxidative deterioration in vegetable oils during the frying process and scavenge free radicals and ROS. Vegetable oils are thus important in the functional and sensory aspect of food products. The oil acts as a medium for heat transfer and as a carrier for the fat-soluble vitamins A, D, E, and K.

Enzymatic and non-enzymatic antioxidants ensure the balance of ROS level and repair oxidative cellular damage. Enzymatic antioxidants such as superoxide dismutase, catalase and glutathione peroxidase, which are directly involved in the neutralization of ROS, are known as the first line defense system [16,17]. On the other hand, the second line of defense is represented by non-enzymatic radical-scavenging antioxidants, which include ascorbic acid, carotenoids, tocopherols and plant phytochemicals such as phenolic compounds (polyphenols) that inhibit the initiation of the oxidation chain and prevent chain propagation [18,19]. Natural polyphenols include phenolic acids and flavonoids [20]. These antioxidants protect cells and biomacromolecules against the harmful effects of free radicals and prevent oxidative degradation.

Frying remains as one of the most popular culinary methods globally, for both industrial and domestic food preparation procedures. Organoleptic and sensorial properties of fried food products, such as juicy taste, nice flavor, crispy texture and brownish color, are largely desired and relished by consumers [21]. However, reheating of the vegetable oil at high temperatures leads to oxidation, which produces rancid odor and flavor [22,23]. Subsequently, the oxidation process reduces both the nutritional value as well as the safety of fried food products through the formation of secondary products due to peroxidation of polyunsaturated fatty acids (PUFAs) [24,25]. The extent of oil degradation is measured by the peroxide index. The peroxide index evaluates the amount of peroxides formed in the vegetable oil during the oxidation process. The extent of oxidation rancidity is influenced by the number of frying episodes. The more frequently the vegetable oil is reheated, the higher is the peroxide index [26,27]. The chemical stability of the frying oil is

influenced by peroxide formation. A higher peroxide value indicates lower chemical stability of the oil.

Increasing the heating temperature and duration may alter the antioxidant activity in the vegetable oils [13]. Heating causes changes in the physical and chemical characteristics of the oils. Repeatedly heating the oil leads to the degradation in the oil quality, with formation of more saturated compounds such as hydroperoxides, monomers, dimers, trimers and high-molecular-weight compounds along with less proportion of unsaturated fats. Lipid peroxidation may be initially prevented by antioxidants. However, repeated heating eventually decreases the antioxidant content of the oil. As a consequence, the remaining depleted antioxidants in the oil will not be capable of exerting any protective effect against free radicals and oxidative damage.

Endogenous antioxidants contained in vegetable oil provide a natural resistance to oxidative deterioration. The antioxidant activity of the phenolic extract of virgin olive oil was found to be very low after the sixth frying process [28]. Cooking oil is more susceptible to oxidation following repeated heating due to the increased concentrations of polar compounds, and oxidized triacylglycerol monomers, dimers and polymers [28]. Similarly, total loss of antioxidant activity due to deep-fat frying after the 12th frying processes has been reported [28]. Vitamin E consists of tocopherols and tocotrienols isomers, which are the major antioxidants of vegetable oils [29]. Adam et al. [30] reported that reheating palm and soybean oils significantly reduced the content of the various vitamin E fractions. The stability of the vitamin E isomers varies during heating because it depends on the type of oil and the content of vitamin E in those edible oils. Palm oil is rich in tocopherols and tocotrienols. Tocotrienol has been exhibited to have more potent antioxidant activity [31,32] than tocopherol, which is found in soybean oils. In addition, soybean oil is high in PUFA content compared to palm oil, which has approximately 1:1 ratio of saturated and unsaturated fatty acids with lower PUFA levels. Hence, soybean oil is more prone to oxidation than palm oil following repeated heating [33].

Deterioration of natural antioxidant such as phenolic compounds and tocopherols is observed when virgin olive oil and sunflower oil are heated repeatedly [34]. Evuen et al. [35] conducted a study to investigate the toxicological effects of heating of vegetable oils on their natural antioxidant levels. The oils were repeatedly heated for three consecutive days. Refined, deodorized palm olein, groundnut oil, congealed and locally made vegetable oil samples showed a reduction in alpha-tocopherol and beta-carotene levels as the frying oils were repeatedly heated [35]. The effect of antioxidants on the stability of rapeseed oil during heating at 80°C and during deep-fat frying were evaluated by determination of the production of polymers, its peroxide index and tocopherol content [36]. Repeated heating reduced the stability of the rapeseed oil, with a lowering of the tocopherol content and an elevation in the levels of lipid peroxidation products. A study carried out by Koh et al. [37] demonstrated that with increased frying cycles, antioxidant activities reduced significantly in palm oil and rice bran oil. Tocotrienol and tocopherol concentrations decreased in both vegetable oils. However, it was reported that tocotrienol is more susceptible to degradation when compared to tocopherol. Both vitamin E homologues are potent antioxidants. Nevertheless, tocotrienol was shown to possess greater antioxidant capacity [31,32].

Hence, it might be less stable and be oxidized first to protect the other antioxidant, i.e. tocopherol.

Effect of reheated vegetable oils on lipid peroxidation

Excessive free radicals cause alterations in the redox state of human body, leading to lipid peroxidation. Although lipid peroxidation is a natural process, unabated, it is a crucial step in basic deteriorative mechanisms that include cell injury, enzyme damage and nucleic acid mutagenesis [38,39]. Lipid peroxidation is one of the key mechanisms causing oxidative modification of physiologically important lipids in cell membranes. Lipids, particularly PUFAs, are key targets of this modification because they contain oxidizable double bonds [40]. The basis for this is the hydrogen adhering to the carbon atom between two adjacent double bonds is the weakest bond in the fatty acid, which makes it susceptible to oxidative attack. Unstable free radicals readily stabilize themselves by abstracting electrons from membrane lipids to initiate a self-propagating chain reaction. Structural rearrangement of the lipids ensues, and the rate of bond cleavage is greatly increased until the molecule is stabilized.

Oxidative damage to lipid architectures can ultimately lead to disorganization and dysfunction of, as well as damage to membranes, enzymes and proteins [41]. Subsequently, lipid peroxidation impairs the membrane functions, inactivates membrane-bound receptors or enzymes, and disturbs ions permeability and fluidity, which eventually leads to membrane rupture [42]. Moreover, reactive electrophilic end products of such lipid peroxidation reactions, namely α - and β -aldehydes are also detrimental to cell viability [43]. Lipid peroxidation provokes alteration in gene expression and immunologic responses [44]. Oxidative damage may accumulate over time, thereby contributing to cell injury and pathologies, including cardiovascular diseases [45,46] and inflammatory disorders [47,48].

As various oxidative reactions are initiated by thermal treatment, the antioxidant defense system of the body appears to be actively challenged by the free radicals present in reheated oils [49]. A previous study has found a higher content of oxidized compounds in the body fat of rats fed oxidized soybean oil [50], suggesting the important role of reheated oil in altering the redox steady state. Depletion of the natural antioxidants, such as phenolic compounds [51], tocopherols and tocotrienols [30] of cooking oil further renders cell membranes vulnerable to lipid peroxidation. Moreover, some end products of oil deterioration such as ketones, alcohols and aldehydes are cytotoxic, the ingestion of reheated oil may lead to cell necrosis and apoptosis [52].

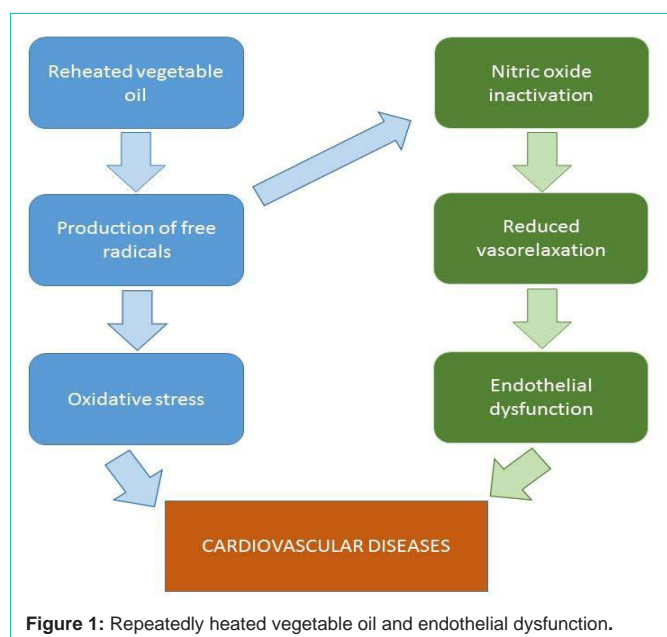
Various techniques are available for the detection and measurement of lipid peroxidation, which include measurement of unsaturated fatty acids levels, estimation of conjugated dienes in lipoprotein fractions, quantification of lipid hydroperoxide and F_2 -isoprostane radioimmunoassay. The thiobarbituric acid reactive substances (TBARS) assay is most commonly used to quantitate malondialdehyde, which is the end product of lipid peroxidation. Generally, consumption of reheated oil increases lipid peroxidation in both animal and human models. Adam et al. [53] found that ingestion of reheated soybean oil exacerbated the lipid peroxidation induced during the post-menopausal stage in rats. The result suggests that thermal treatment generates free radicals in oil, which enhance oxidative stress in the animals. Similarly, post-prandial oxidative

Table 2: Effect of reheated vegetable oils on lipid peroxidation.

Study	Reheated oil	Diet formulation	Subject	Duration	Results
Corcos et al. [69]	Soybean	15% of oil in diet	Young and aging rats	10, 90, 180 and 365 days	TBARS ↑ (with earlier effects in aging rats)
Hageman et al. [70]	Coconut PUFA-rich vegetable frying oil	10% w/w of oil in diet	Male rats, inbred strains	4 weeks	TBARS slightly ↑ by PUFA-rich oil; ↓ by coconut oil
Staprāns et al. [71]	Oxidized vitamin E-depleted corn oil	1 g/kg body weight	Male volunteers	An 8-hour period	TBARS ↑ Conjugated diene in chylomicrons ↑
Staprāns et al. [72]	Vitamin E-depleted corn oil	5% of oil in 0.25% cholesterol diet	New Zealand white rabbits	12 – 14 weeks	Conjugated diene in β-VLDL ↑
Eder [73]	A mixture of lard and safflower oil (2:1 w/w)	10% of oil in diet	Male Sprague-Dawley rats	35 days	Total MUFA/SFA ratio ↓
Quiles et al. [74]	Olive Sunflower	80 g/kg diet	Male Wistar rats	8 weeks	TBARS ↑ Hydroperoxides ↑ MUFA ↓ (reheated sunflower only)
Eder et al. [75]	A mixture of sunflower and lard (1:1 w/w)	100 g/kg oil in semisynthetic diet	Male Sprague-Dawley rats	8 and 9 weeks	Susceptibility of LDL to copper-induced lipid peroxidation ↑
Garrido-Polonio et al. [76]	Sunflower	15 g/100 g diet	Male Wistar rats	27 days	Liver, serum, HDL, LDL and VLDL-TBARS ↑
Adam et al. [53]	Soybean	15% w/w of oil in 2% cholesterol diet	Estrogen-deficient rats	16 weeks	TBARS ↑
Yen et al. [77]	Soybean	10% of oil in diet	Male SHR and WKY rats	10 weeks	TBARS ↑ 8-iso-prostaglandin F _{2α} ↑
Leong et al. [27]	Palm	15% w/w of oil in diet	Male Sprague-Dawley rats	6 months	TBARS ↑

Symbols indicate the following: ↑, increased; ↓, decreased; ↕, no changes

Abbreviations are: HDL: high-density lipoprotein; LDL: low-density lipoprotein; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid; SHR: spontaneously hypertensive rat; TBARS: thiobarbituric acid reactive substances; VLDL: very low-density lipoprotein; w/w: weight/weight; WKY: Wistar-Kyoto



stress after the intake of reheated oil has also been reported in human subjects [54]. Increased oxidative stress in human may lead to lipid peroxidation, which subsequently impairs endothelial function in the regulation of vasomotion [55]. Impacts of reheated oil on lipid peroxidation have been documented in Table 2. All of these results demonstrate that thermally oxidative modification of the fatty acid composition in diet may increase cell susceptibility to lipid peroxidation.

Effect of reheated vegetable oils on endothelial function

In addition to being the physical barrier between vessel wall and the blood, the endothelium is an important structure that possesses

both endocrine and paracrine functions. Furthermore, the endothelial cell is able to respond to physical and chemical signals that regulate vascular tone, cellular adhesion, platelet aggregation, smooth muscle cell proliferation and inflammation [56,57]. Vasomotion by the endothelium is responsible for the balance of tissue oxygen supply and metabolic demand by regulation of vascular tone and diameter, in addition to being involved in the remodeling of vascular structure and long-term organ perfusion [58]. Measurement of endothelial function has become an important means to detect arterial abnormalities and represents an early marker of cardiovascular diseases.

When exposed to deep-frying temperatures, fatty acids in the vegetable cooking oil undergo chemical configurational changes from *cis* to *trans* isomers. In addition, generation of oxidized products due to the reheating process leads to a deleterious effect on the vascular function. Nitric oxide (NO), which is also known as endothelium-derived relaxing factor, is released by the endothelium to regulate homeostasis of the vascular system to preserve its integrity. NO causes vascular smooth muscle relaxation through cyclic guanosine monophosphate. Endothelial dysfunction is associated with abnormal endothelium-dependent relaxation. Previous research findings in our laboratory clearly showed that repeatedly heated palm oil and soybean oil cause impairment in endothelium-dependent vasorelaxations and augmentation of contractile responses in adult male Sprague-Dawley rats [33]. Similarly, it has been documented that long-term intake of thermally oxidized palm oil alters the function of aorta isolated from the rat [59]. This indicates an increase in vascular reactivity, which would contribute to increasing vascular tone, eventually elevates blood pressure levels. Similarly, intake of repeatedly heated oil was observed to produce harmful effects on endothelial function in normal young healthy volunteers when they were given heated olive, soybean or palm oils that had undergone either 10 or 20 deep-frying rounds [60].

In a study by Williams et al. [55], ingestion of a meal rich in fat previously used for deep-frying in a commercial setting resulted in impaired arterial endothelial function in healthy men. Their findings suggest that intake of deteriorated products of heated dietary oil may contribute to endothelial dysfunction. Plotnick et al. [61] reported that pre-treatment with the antioxidant vitamin C and E is able to restore endothelial function, suggesting an oxidative mechanism. In our earlier studies [3,4], consumption of repeatedly heated vegetable oil has been shown to significantly reduce NO levels in rats. Reheating of vegetable oil promotes oxidative stress, causing NO sequestration and inactivation. The ability of endothelial cells to release NO may be down-regulated in the presence of oxidized low-density lipoprotein cholesterol and oxidative stress. Peroxynitrite, generated from the reaction between NO and ROS, is a potent pro-oxidant that may play a role in the development of endothelial dysfunction. Reduced endothelium-derived NO bioavailability further enhances contraction of vascular smooth muscle. Thus, consumption of repeatedly heated vegetable oil leads to endothelial dysfunction (Figure 1).

Conclusion

Long-term intake of diet comprising reheated vegetable oil leads to endothelial dysfunction. Repeatedly heated dietary vegetable oil promotes oxidative stress, resulting in NO inactivation and reduced bioavailability. Moreover, antioxidant effect of fresh vegetable oil against free radicals may be reduced gradually as the oil is repeatedly heated. Production of free radicals and reduction of antioxidant and vitamin levels eventually lead to oxidative stress. Oxidative stress and endothelial dysfunction play pivotal roles in the pathogenesis of cardiovascular diseases, which may be controlled by diet modification. Ingestion of repeatedly heated vegetable oil should be restricted due to the detrimental consequences on health.

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