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DETERIORATIVE EFFECTS OF REPEATED BOILING ON THE NUTRITIVE VALUE OF EDIBLE OILS

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ABSTRACT

The deteriorative effect of high temperature exposure to the quality of food grade oils is a well-established fact. However the toxic effects of the end products of thermal oxidation on the natural nutritive value that the oil possesses is not clearly known. So the present exercise is an attempt to understand the toxic effects of repeated boiling, a major culinary practice seen in many parts of the world. Fresh oils and oils stored for long periods in inappropriate conditions are being employed as comparators. Frequently used food grade oils, divided into three categories i.e. fresh, long stored and overcooked were analyzed for their normal lipid quality indices; primary, secondary and total oxidation status and total phenolic contents. Deterioration rate of overcooked oils was found to be almost comparable to that of long stored oils. A significant decline of the total phenolic content observed in the overcooked oils has been represented as loss of nutritive value.

Key Words: Food grade oils, Overcooked, Long stored, Thermal oxidation, Total phenolic content.

INTRODUCTION

Edible oils and fats constitute one of the three major components of a daily diet, the other two being carbohydrates and proteins. They are high energy suppliers amongst all the dietary constituents, and food grade oils in particular play an important role in the body as carriers of essential fatty acids (EFA). As EFAs are not synthesized in the body, they need to be supplemented through the diet to perform various crucial biochemical functions such as maintenance of the cell membrane integrity, synthesis of important hormones like prostaglandins and control of

many physiological factors viz., blood pressure, cholesterol level, and the reproductive system (Rizwana S, 2008, Aparicio R and Aparicio-Ruiz R, 2000, Shahid I and Bhanger 2007). From a chemical perspective, food grade oils are complex mixtures containing a broad range of major components like triacylglycerols (TAGs) and minor compounds representing different varieties of molecules, such as free fatty acids (FFAs), diacyl glycerides (DAGs), wax esters, alkanes, sterols, oxidized TAGs, hydrocarbons, tocopherols, tocotrienols, phenolic compounds, phospholipids and triterpenic acids. (Jun-Cai H *et al.*, 2012; Rosaria C and Beatrice De G, 2011; Gaye Y, 2009; Justyna G, Waldemar W, 2011; Nyama KL, *et al.*, 2009; Nikokavoura *et al.*, 2011).

It is a well-established fact that the food grade oils are susceptible for various physical and chemical stress

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conditions, which can have a serious effects on the quality (color, flavor and texture) and nutritive value of the oil there by causing serious health hazards to the consumers (Chinu C and Thankappan R, 2011). The major route for the spoilage of any food grade oil is understood to be lipid oxidation, which is a free radical chain reaction generating free radicals, which are the major causes for the deleterious effects. The oxidative effects on the oil can be influenced by the fatty acid composition of the oil, the processing strategy used during its manufacture (Bertrand M *et al.*, 2010; Dhellot JR *et al.*, 2006), influence of heat and light, storage method employed for the oil, different culinary practices (boiling, microwave heating, baking, and deep frying) and most importantly the exposure of oil to atmospheric oxygen (Gaye Y, 2009; Jae Min L *et al.*, 2007; Noelia T *et al.*, 2009). In addition, the factors do have their effects on the reduced shelf life of the oil; making it a serious issue of concern for the food industry.

In recent years, due to the enhanced awareness for a healthy diet, increased attention is been paid towards assessing the quality of the oil that's used for cooking. Assessing the oxidation level of oil is becoming an important quality criterion for food industry. Even though there is a considerable amount of information available on the nutritional aspects, quality assessment and the nutritive value deteriorating effects of different chemical and physical factors with respect to extra virgin olive oil (EVOO) which is a commonly used cooking fat in Mediterranean countries (Manuel *et al.*, 2009; Noelia T *et al.*, 2009; Gaye Y, 2009; Antonia C, *et al.*, 2009; Eleni PL *et al.*, 2001; Lukas *et al.*, 2009; Vlachos N *et al.*, 2006; Manuel B *et al.*, 1999) there is a void of such information available with respect to many other food grade oils used in Asian countries. Thus the present work is an attempt to look at the quality and nutritive value deteriorating effects of repeated boiling, unfortunately a widely seen common culinary practice; on the frequently used food grade oils like groundnut oil, sunflower oil, gingelly oil and coconut oil. To be able to compare and understand the exact effects of repeated boiling on the chosen parameters, fresh and long stored samples of the respective oils are used as positive and negative comparators respectively.

MATERIALS AND METHODS

Apparatus and chemicals

Absorption measurements were taken using UV-Vis spectrophotometer (UV-1700 Pharma Spec – Shimadzu).

All the chemicals used were of analytical purity. Acetic acid, Potassium iodate, , Diethyl ether, Folin-Ciocalteu reagent, Gallic acid, Tween 20, Chloroform, Isooctane, p-Anisidine, Acetonitrile were procured from SDFCL; Sulfuric acid was procured from QUALIGENS; Ethanol was procured from Chenyshu Yangyuan Chemicals and Potassium iodide, Sodium thiosulphate, Starch, Phenolphthalein, Potassium hydrogen phthalate,

Potassium hydroxide, Methanol and Sodium carbonate were procured from MERCK.

Samples and sample preparation

Fresh, unadulterated and refined food grade oils; Groundnut oil (GNO), Sunflower oil (SFO), Coconut oil (COO) and Gingelly oil (GIO) procured from the local oil mills were used for the analysis. Care was taken to make sure that the oils at the time of collection were very fresh. The oils were subdivided into three categories each, viz. Category I – Fresh oil, Category II – Long stored oil and Category III – Over cooked oil. Samples for Category I were the fresh oils themselves. Category II samples were obtained by storing the requisite aliquots of the freshly procured oil samples for a period of 4 – 5 months in loosely tightened plastic bottles, giving an easy access for the atmospheric oxygen. The oils were stored until there was a visible change in their color, thickness and aroma. Samples for Category III were obtained by repeatedly boiling (3-4 times) the oils on electric hotplate maintained at 100°C, until there were considerable physical changes in the oils as mentioned above.

Chemical analysis

Lipid quality indices: Basic lipid quality indices like Saponification value (SV), Iodine value (IV) and Free fatty acid / Acid value (AV) were determined using the official methods AOAC 920.160,1997 - for the determination of SV, AOAC 993.20,1997 – for the determination of IV and AOAC 969.17,1997 - for the determination of AV. All the procedures were carried in triplicates.

Analysis of Primary, secondary and total oxidation of oils

Peroxide value (PV): Official Method of Analysis (European Commission Regulation EEC N-2568/91) was employed for determination of PV. Accordingly, 10 ml of chloroform, 15 ml of acetic acid and 1 ml of potassium iodide solution were added into 3 g of an oil sample taken in a stoppered volumetric flask, and mixed vigorously for 1 minute. Then, the sample was kept in dark at room temperature for 5 minutes. Later, 75 ml of deionized water and 0.5 ml of starch solution were added to the oil sample. Titration of free iodine was carried out with 0.002 M standardized sodium thiosulphate solution until the dark blue color of solution turns to colorless and the amount of total sodium thiosulphate solution rundown was recorded. Tests were carried out in triplicates and a blank was carried without the oil sample. PV was calculated using the expression:

$$PV(\text{meq Kg}^{-1}) = \frac{(V - V_0) \times M \times 1000}{W}$$

Where V is the titre value (ml) of sodium thiosulphate for sample, V_0

is the titre value (ml) of sodium thiosulphate for blank, M the molarity of sodium thiosulphate and W is the weight of oil sample (g).

p - anisidine value (p-AV): Standard IUPAC method (IUPAC - 2504, 1987) was employed for the determination of p-AV. Accordingly, in triplicates, 100 mg of the oil sample was weighed into a 25 ml volumetric flask and was dissolved and diluted to 25 ml with isooctane. Absorbance of the solution was measured at 350 nm using UV – Visible spectrophotometer with isooctane solvent as a blank. Later 2.5 ml of the above solution was mixed with 0.5 ml of p-anisidine reagent (0.5 % w/v p-anisidine in acetic acid), incubated exactly for 10 minutes and then the absorbance was measured at 350 nm, using the p-anisidine reagent as a blank. AV was calculated using the expression:

$$p - AV = 25(1.2 X A_2 - A_1)/W$$

Where A_2 is the absorbance of solution prepared with p-anisidine analytical reagent, A_1 is the absorbance of solution prepared with isooctane and W is weight of the sample (g).

Totox value (TV): TV of the oil, which is an indicator of the total oxidation the oils have undergone, was measured using the method mentioned by Fereidoon Shahidi and Ying Zhong; 2005. It was calculated using the expression:

$$Totox\ value = 2PV + p - AV$$

Where PV is the peroxide value and p-AV is the p-anisidine value of the respective oils.

Total phenolic content (TPC): TPC of the oil samples were determined by the Folin–Ciocalteu spectrophotometric method with gallic acid as a reference phenolic standard (Gaye Yildirim, 2009). Extraction of phenolic compounds was done by taking 2g of an oil sample to which 10 ml of methanol/water solution (80:20

v/v) and 1-2 drops of Tween-20 were added and homogenized with a blender for 1 minute and then centrifuged at 5000 rpm for 10 minutes. After centrifugation the supernatant was collected in a tube and the extraction with the remaining residue was repeated two more times without Tween 20. All the supernatants were pooled at the end of extractions and the volume was recorded. Extracts for all the categories of oil samples were prepared in the same procedure.

In triplicates, to 1 ml of each extract, 1 ml of the methanol/water solution (80:20 v/v) was added and diluted with 5 ml of deionized water. Later 0.5 ml of Folin-Ciocalteu reagent, 2 ml of sodium carbonate solution (15 % w/v) was added and the mixture was diluted with 1.5 ml of deionized water. The tubes were vortexed for 30 seconds and kept in dark for 2 hours. Blank was prepared in the same procedure with 1 ml of methanol-water (80:20 v/v) instead of phenolic extract. Finally the absorbance was measured at 765 nm in a UV-Visible spectrophotometer. The total phenolic content of each extract was determined by using a gallic acid calibration curve which was constructed using standard gallic acid solution prepared with different concentrations ranging from 15 mg/L to 300 mg/L. total phenol content was determined as mg gallic acid / kg oil. TPC was calculated using the formula,

$$TPC = \frac{GA \times V \times 1000}{W}$$

Where GA is the gallic acid concentration (mg/ml) from the standard curve, V is the total volume (ml) of the extracts pooled and W is weight of the sample (g).

RESULTS AND DISCUSSION

Lipid quality indices

The lipid quality indices of different categories of oils are presented in Table 1.

Table 1. Lipid quality indices of different categories (I / II / III) of edible oils (GNO / SFO / COO / GIO)*

Parameter	GNO			SFO			COO			GIO		
	I	II	III	I	II	III	I	II	III	I	II	III
SV(mg of KOH/g of oil)	291±1.46	324.2±1.15	303.5±1.10	75.2±0.87	143.8±0.85	102.2±1.40	298.4±1.11	412.2±0.95	356.6±0.59	38.7±0.46	123.9±1.57	61.6±1.43
IV (g of I ₂ /100 g of oil)	11.34±0.05	21.81±0.28	12.79±0.14	16.3±0.13	31.49±0.05	25.21±0.07	1.72±0.05	2.15±0.04	10.64±0.08	13.79±0.16	21.59±0.05	14.86±0.05
AV(% oleic acid)	2.28±0.04	4.28±0.05	3.69±0.06	4.68±0.03	6.61±0.02	5.37±0.05	5.38±0.04	9.53±0.05	5.58±0.06	5.76±0.04	6.47±0.06	5.90±0.05

* Mean ± Standard deviation

The percentage rise in the SV of long stored (category II) and over cooked oils (category III) compared to the fresh oils (category I) are GNO (11%, 4%), SFO (90%, 36%), COO (38%, 19%) and GIO (223%, 60%) respectively signifying the enhanced loss of freshness both in the long stored and over cooked oils. A similar trend of rise in the IV can be observed, where in the percentage rise amongst the category II and category III compared to category I are GNO (90%, 9%), SFO (93%, 56%), COO (100%, 900%) and GIO (61%, 7%) respectively. The IV

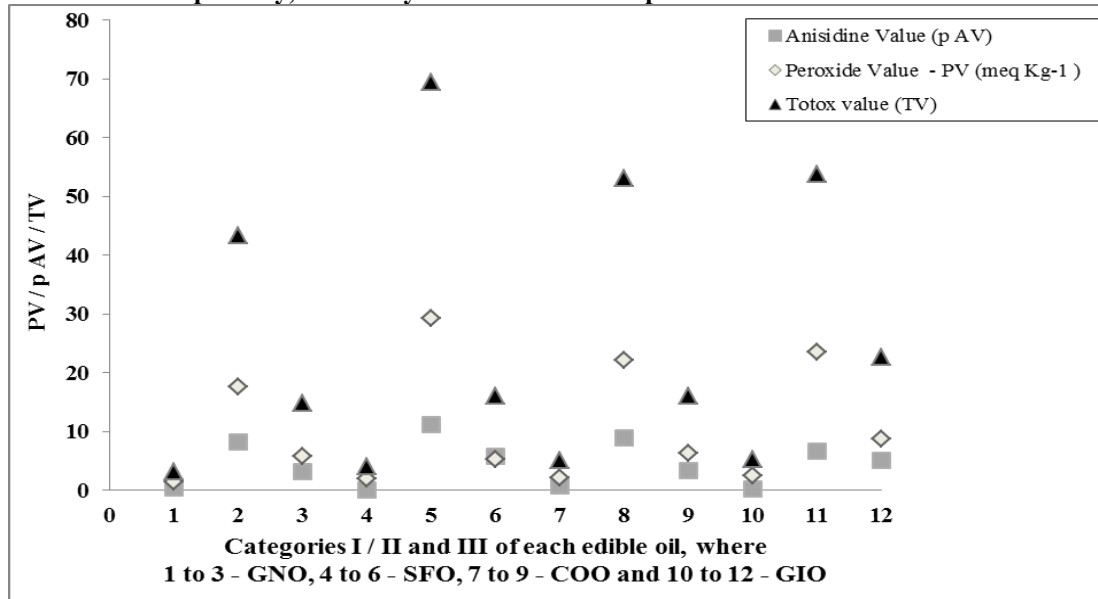
reveals the enhanced degree of unsaturation which is much more in long stored oils as expected, but is also considerably high in the overcooked oils. The trends in SV and IV are in support with the rise in the AV, which as expected increased during storage. The percentage rise in AV amongst the category II and category III compared to category I are GNO (100%, 50%), SFO (100%, 50%), COO (80%, 3%) and GIO (20%, 2%) respectively.

Analysis of the primary, secondary and total oxidation of oils

Since it is evident that the overcooked oils are showing a rise in the degree of unsaturation at a rate almost

comparable to the long stored oils, the further attempts were to look at the extent of oxidative damage the oils have undergone. The extent of primary, secondary and total oxidation of the oils is presented in Graph 1.

Graph 1. Assessment of the primary, secondary and total oxidation patterns in the edible oils of different categories

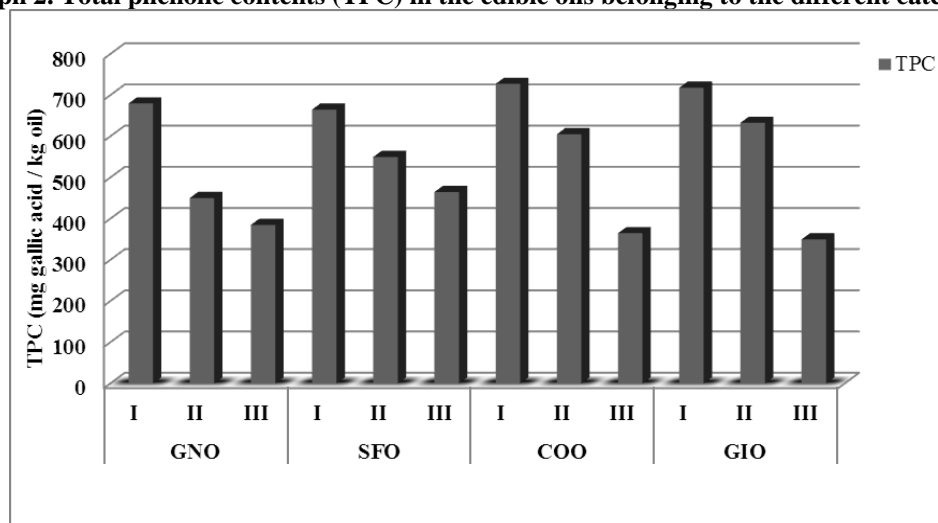


PV and p-AV which are indicator of primary and secondary oxidations respectively are almost negligible for the category I oils, proving that the oils are fresh. There is a gradual rise in both the values with respect to the category II oils as a result of the storage conditions the oils were subjected. A similar kind of rise in the indices can be observed in category III as a result of the oxidative damage the oils have undergone due to heating. The results are in strong correlation with the observations made by many other works, trying to look the effects of different culinary

practices on the oils (Antonia C *et al.*, 2009; Bertrand M *et al.*, 2010; Chinu C and Thankappan R, 2011; Noelia T *et al.*, 2009; Eleni P, *et al.*, 2001; Lercker G, Rodriguez-Estrada MT, 2000).

Total phenolic content: To look at the dietary safety aspects of the consumption of overcooked oils, changes in their total phenolic contents in comparison to the negative and positive comparators i.e., fresh and long stored oils were analyzed and the results are presented in Graph 2.

Graph 2. Total phenolic contents (TPC) in the edible oils belonging to the different categories

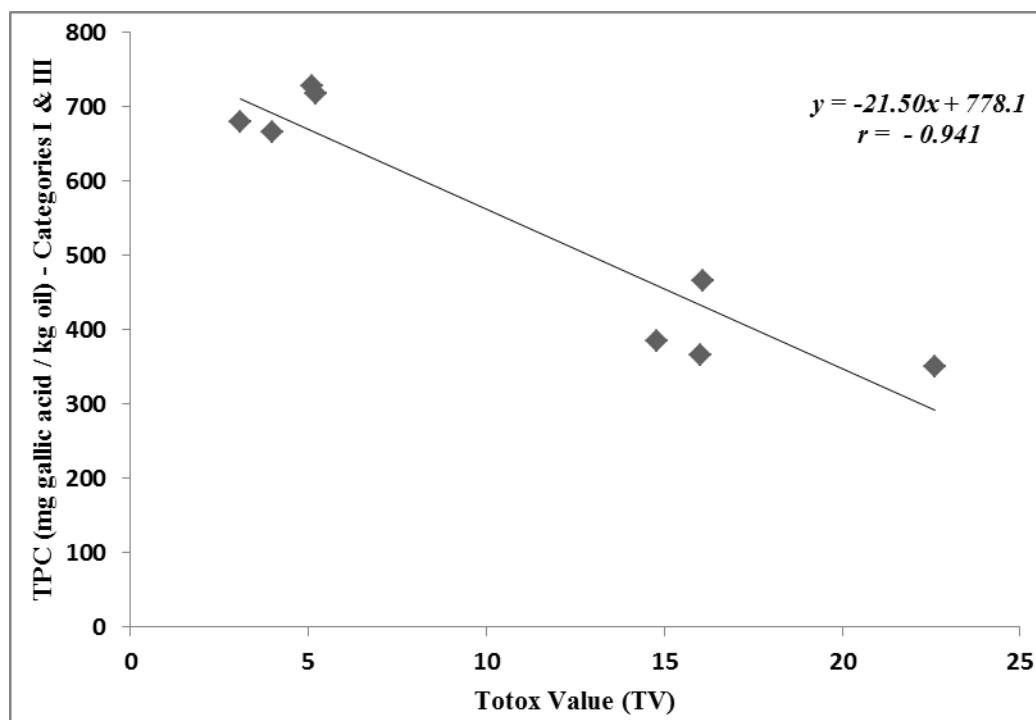


The total phenolic content significantly decreased both in the long stored and overcooked oils compared to the fresh oils. The percentage decrease in the TPC values of category II and III compared to category I are; GNO (33%, 43%), SFO (17%, 30%), COO (24%, 49%) and GIO (11%, 51%) respectively.

Correlation between TV and TPC: There exists a strong

negative correlation between the TV and TPC with a correlation coefficient (r) value of -0.941 which is a significant observation establishing the fact that TPC decreases with a rise in TV which is a product of both primary and secondary oxidations the oils have undergone and thereby indicating the loss of its nutritive value (Graph 3)

Graph 3. Correlation between the total phenolic contents and totox values of the four edible oils especially categories I and III.



CONCLUSION

Upon heating edible oils are known to undergo chemical reactions which majorly include hydrolysis, oxidation and polymerization accompanied by the release of an array of degradation products such as free fatty acids, hydroperoxides and polymerized triglycerides. The amount of degradation products increases with the duration of heating at high temperatures and some of these may be used to indicate the degree of degradation of edible oil. The toxicity of these degradation products is of health concern.

Autooxidation is known to be the most common process leading to oxidative deterioration of food grade oils with high unsaturated fatty acid contents (Prasant, *et al.*, 2011). It is generally a spontaneous reaction of atmospheric oxygen with the lipids and the process is found to be accelerated at higher temperatures (thermal oxidation), such as those experienced during deep-fat frying (Alireza S *et al.*, 2010; Antonia *et al.*, 2009; Frega N *et al.*, 1999), resulting with a profound rise in free fatty

acid and polar matter contents, foaming, color, and viscosity of the oil (Fereidoon S and Ying Z, 2005). The chosen parameters, PV and p-AV are known to reflect the extent of oxidation at early and later stages of oxidative deterioration of oils respectively. TV measures both hydroperoxides and their breakdown products in total and provides a better estimation of the progressive damage that the oil is undergoing (Fereidoon S and Ying Z, 2005). Though the cooking oils are isolated majorly from plants and are naturally expected to be rich in antioxidants (Adel A *et al.*, 2010; Proestos C *et al.*, 2005; Karina, *et al.*, 2006), it is proved that the intrinsic antioxidant capacity that the oils possess is inadequate to preserve them especially during their exposure to high temperatures and oxygen which occur during cooking and storage (Sabrina CM *et al.*, 2007).

The extent of damage caused by repeated boiling on the organoleptic and nutritive properties of category III oils is found to be almost comparable to the damage caused to the category II oils, which is quite an important

observation. Though the further characterization and quantification of the degradative toxic end products would give a better understanding of the boiling induced structural and functional changes in the nutritive contents

of the edible oils, the present work certainly signifies the necessity of avoiding the usage of repeatedly boiled food grade oils as they are losing their inherent nutritive value and would even be toxic if consumed.

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