Study the Change in the Molecular Structure and Toxicity of Processed Frying Oils, in Vitro

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Abstract

In the present work, we studied the cytotoxic effect and the change in the molecular structure of processed frying sunflower oil samples. The cytotoxicity results showed high level of peroxidase content in the cells exposed to the processed frying oils than the control one, at the same time The IR spectrum of the oil samples showed some variations in the intensity of some groups which; indicates a change in the molecular structures of the oil samples after used in frying food. The results given by the FTIR spectrums is greatly supported by measuring the cytotoxicity in the biological cells exposed to the different samples of sunflower oil individually.

Keywords

Cytotoxicity, sunflower oil and Fourier transform infrared (FTIR) spectroscopy

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Introduction

The consumption of the Processed seed oils like sunflower oil had been increased lately with so many questions about their possible biological hazards on human cells., However that the main stream nutrition organization recommended using them. These oils hold high amounts of biologically dynamic fats named Omega-6 polyunsaturated fatty acids, which are hurtful when secreted in extra amount [1]. Fatty acids are very vital for human body and because our body can’t produce them it is very important to get them from a balanced diet; There are two main types of fatty acids: The Omega-3 and Omega-6 fatty acids. These fatty acids also support vital functions linked to paths known to affect several systems like the immune system [2] Consequently; the un- balance between Omega-6s and Omega-3s can cause severe harm. Likewise, the relative unsaturation of these fatty acids has a great hazard to human health in such a way that; as the number of the double bonds in a fatty acid increases, its reactivity increases. Polyunsaturated fats tend to interact with oxygen, which can cause chain reactions, damaging

other structures including vital structures like DNA [3,4] These fatty acids tend to affect the cell membranes and increasing harmful oxidative chain reactions. It has been reported that the oxidation of polyunsaturated fatty acids (PUFAs) which caused by heat in foods and cooking oils during standard frying or cooking is a process that produce a wide range of aldehydic products which has an extremely toxic nature [5]. Polymerization, in which rather small molecules of fat or oil combined to form much large molecules, can happen in the deep frying of foods; where frying is done at temperatures ranging from 325° to 375°F. The rate of polymerization rises with the degree of unsaturation which accordingly affect the toxicity of the oil used.

Fourier transform infrared spectroscopy (FTIR) was used for characterizing vegetable oils and studying the effect of repetitive using of frying oil. [6]

In present work FTIR spectroscopy is used to assess the thermo-oxidative variations induced through thermal heating by using vibrations of different chemical groups at specific wavelengths of the spectrum, the variations occurred in IR spectra during heating of sunflower oil have been studied in relationship with the cytotoxicity [7,8]. Some frequencies were known to be useful for evaluating the effect of heat on oil quality as they give evidence regarding the amount of thermo-oxidative degradation. In another word, the use of some ratios of the absorbance of specific bands recorded in the FTIR spectra shows the alteration occurred in cooking oils by thermal stress. [9]

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Materials and methods:

Commercially Sun-flower oil were purchased from the local market. Three types of oil samples were used, sample (1) is the control, sample (2) is the one that had been used only one time in frying one single type of food and the third one (3) is the one that had been used more than one time in frying different types of food. Oil samples were heated under simulated frying conditions, using an electric oven.

Biological sample preparation:

Two cell lines “Vero cells” isolated from African green monkey kidney epithelial cells, and WI-38 cell line extracted from human lung fibroblast (purchased from VACSERA Giza) were harvested from 25 cm^2 culture flasks using trypsinization by the addition of 100 µl trypsin-EDTA (trypsin, 250 mg/L and EDTA, 100 mg/L phosphate-buffered saline). Once the cells were dissociated from the bottom of the flask, 100 µl trypsin-inhibitor (fetal calf serum) was added,

and cells were subsequently added in 96-well culture plates with 200 µl MEM medium counted with a haemocytometer and incubated at 37 °C in 5% CO_2.

MTT cytotoxicity:

MTT (3- [4,5- dimethylthiazol-2-yl-2,5-difeniltetrazolium bromide) is a water-soluble salt of tetrazolium. It is transformed into an insoluble formazan which is accumulated in cytoplasm. The amount of formazan depends on activity of mitochondrial succinate dehydrogenase and is proportional to the number of living cells. The concentration of formazan can be determined by a colorimetric method. The MTT assay was performed basically as described, [10] with some modifications [11]. In brief, medium was aspirated from the wells and freshly prepared MTT (dimethylthiazol diphenyl tetrazolium bromide) phosphate-buffered saline (5mg of /ml) was added. Cells were incubated with MTT for 3 hours in the incubator (37 °C/5% CO2). Subsequently, 100 µl dimethyl sulphoxide DMSO was added to each well after aspiration of the MTT-medium and the plates were shaken for 5 minutes on a microplate shaker. The quantity of formazan was measured in absorbance at 540 nm were read immediately thereafter on an automatic microplate reader (ELX 800 UV (Bio-TEK). The cell viability was determined as the ratio of the optical density (OD) of the sample to the OD of the control solution and [12] expressed as a percentage. If the percentage was greater than 60%, the oil had no cytotoxicity, and if it was less than 60%, the oil was highly cytotoxic. Less than 60% viability are considered high toxic.

Macrophage isolation:

Rats were sacrificed, and blood was collected in heparinized tubes. Leukocytes were isolated from blood, according to Jeney et al, 1997[12]. Briefly, 1 ml of histopaque (Sigma, St., Louis, MO) containing bacto-hemagglutination buffer, pH 7.3 was dispensed into a siliconized centrifuge tube, then 1 ml of anticoagulated rat blood grouped samples was carefully layered on the top. The gradient was centrifuged at 500xg for 20 min at 4°C. The interface of leukocyte suspension was gently collected with a glass Pasteur pipette and dispensed into a siliconized tube, washed twice with phenol red-free Hank' s balanced salt solution (HBSS, Sigma) and adjusted to 2×10^6 viable cells per milliliter.

FTIR spectrum measurement:

IR spectra were measured at room temperature in the range 400–4000 cm^{-1} on Fourier transform infrared spectrometer (FT/IR-6100, Jasco, Japan). All spectra were recorded in the range (4000 –

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400 cm^{-1}), the number of scans was 128, and the resolution was 4 cm^{-1} and scan speed 2 mm /s. All spectra were processed with the computer software program Spectrum.

A film of a small amount of each sample (2 µl) was deposited between two disks of KBr, avoiding the presence of air. For each sample, the spectra were recorded 3 times.

Results

Cytotoxicity measurement:

The toxic effect of the frying sunflower oil samples on both African green monkey and human fibroblast is shown in figure 1

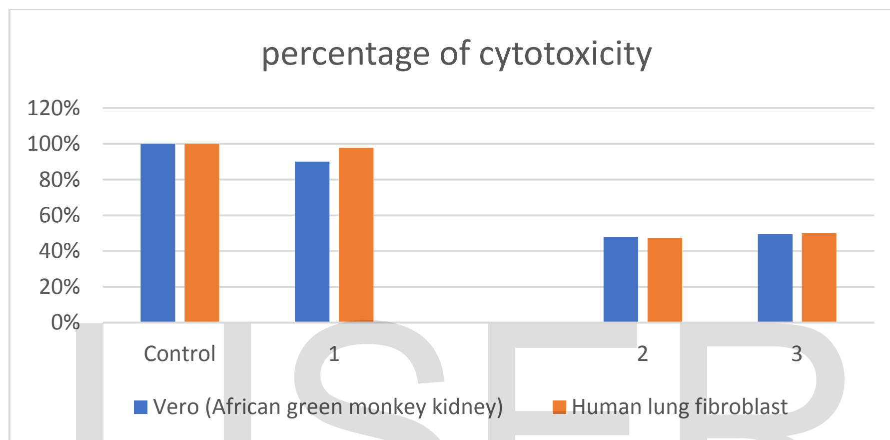

Figure 1: Shows the percentage of cytotoxicity

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Macrophage cytotoxicity:

The percentage of the toxicity of the heated sunflower oil samples on macrophage cell production is shown in table 1.

control	Oil Sample 1	Oil Sample 2
1.347	0.976	0.792
63.3%	45.9%	37.2%

Table1: Shows the percentage of macrophage toxicity

The results from figure 1 shows that the cytotoxicity of both kinds of cells that exposed to heated vegetable oils (after frying food) is highly increased than the one exposed to the control oil. also, the degree of toxicity slightly depends on the cell type (toxicity towards African green monkey kidney cells is slightly higher than human lung fibroblast while the Macrophage cells in table 1 shows the highest toxicity towards the processed heated oils.

FTIR Spectrum

FTIR spectra of control oil and frying oil are shown in Fig. 1. Careful examination of the FTIR spectra of control oil and frying oil indicated that there are no differences between their spectra except minor changes in the absorbances of some bands.

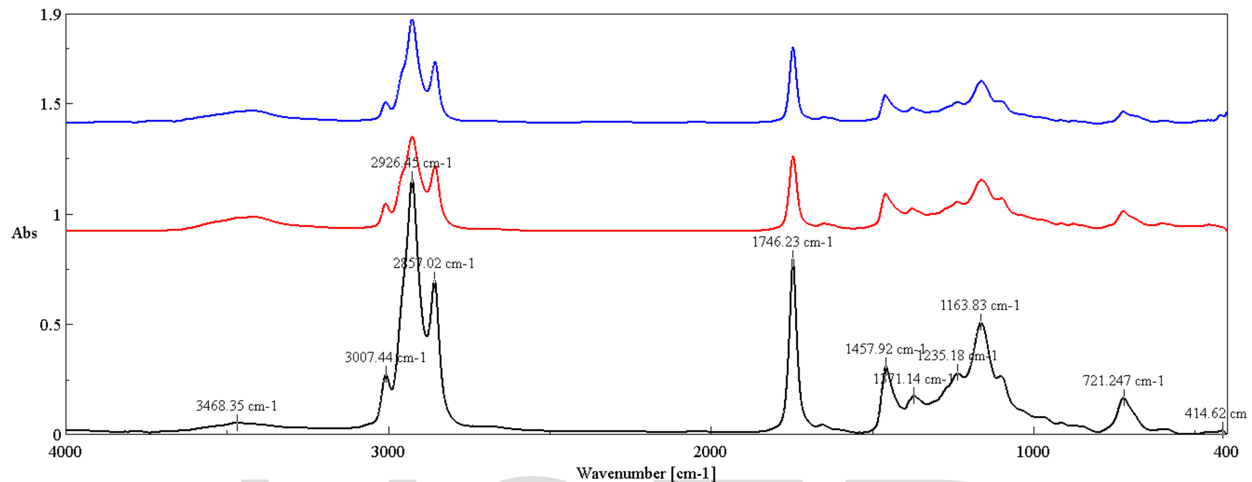

Figure 1. FTIR spectra of oil samples

sample 1 (—), sample 2 (—) and sample 3 (—)

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The results showed that All spectra had absorption bands at wavenumber 3006 cm^{-1} which are assigned to symmetric vibration of = CH, the band at 2954 cm^{-1} attribute to asymmetric vibration of –C–H of CH_3, band at 2924 cm^{-1} assigned to asymmetric vibration of –C–H of CH_2, band at 2854 cm^{-1} is usually referred to symmetric vibration of –C–H of CH_2 and CH_3, the carbonyl band of –C=O appeared at 1746 cm^{-1}, while the band at 1648 cm^{-1} owing to vibration of –C=C–, the band at 1459 cm^{-1} attribute to vibration of –C–H of CH_2,CH_3, 1376 cm^{-1} (symmetric vibration of –C–H of CH_3), the bands at 1236 cm^{-1} and 1162 cm^{-1} (vibration of –C–O, –CH_2–), the band at 1100 cm^{-1} is due to vibration of –C–O), the band at 915 cm^{-1} (vibration of cis –HC=CH–), and the band at 721 cm^{-1} assigned to vibration of cis –HC=CH– bending out of plane).

Table 2: The values of absorbance ratio from IR at aldehyde group for sunflower oil.

Absorbance	1745/2924	1745/2855	1163/1099
Sample 1	0.9344	1.0899	0.645
Sample 2	0.871	1.1053	1.487
Sample 3	0.831	1.1685	1.725

Table 2 shows the values of absorbance ratio recorded from IR at specific wave numbers of aldehyde group for frying sunflower oil.

Discussion

Lipid peroxidation is a process that occurred naturally in the body, because of producing several reactive oxygen species (hydroxyl radical, hydrogen peroxide etc.) in a small amount due to normal process of energy production in mitochondria. It can also be produced by the act of lot of phagocytes; when secreted in excessive amount, These reactive oxygen species (ROS) attack the polyunsaturated fatty acids of the fatty acid membrane, promoting a self-propagating chain reaction and leading ultimately to the formation of aldehydes [13]. In our present work it can be noticed that there is high decrease in the cell viability and increase in the percentage of cytotoxicity on the different cells exposed to oil samples in such a way that the oil sample 3 in which the oil used in frying different kinds of food more than one time has more cytotoxic effect than the oil sample 2 in which the sunflower oil had been used to fry one kind of food for only once. At the same time oil sample 2 and 3 shows elevated level of cytotoxicity than the control group this might indicate the destruction of membrane lipids in the process of lipid peroxidation and the e formation of the end-products of such lipid peroxidation reactions which are extremely dangerous for the viability of cells, even tissues. [14]. At high concentrations, aldehydes were known to be part of the cause of the toxic effects of lipid peroxidation. Aldehyde toxicity is mostly due to the variations of several cell functions, which usually depend on the formation of covalent adducts with cellular proteins [15]. Due to their amphiphilic nature, aldehydes can simply diffuse across membranes and can covalently modify any protein in the cytoplasm and nucleus, far from their site of origin [16]. Correspondingly, the aldehydes formed outside the cells (i.e., in a site of inflammation or in plasma), can react with adjacent cells, even in cases when they are not primary sites of lipid peroxidation. The goals of lipid peroxidation-derived

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aldehydes are cell-type which depend both on the pattern of proteins expressed by the cell and the aldehyde concentration. these findings can be supported by the results from the FTIR spectra in which there is an alteration in the intensity of some absorption bands, which according to some previous researches [17,18], indicated that the absorbance at 1753 cm^{-1} is characteristic to the oils with a high saturated fatty acids content and short carbohydrate chain.

The decrease was detected for the absorbances ratio A1746 cm^{-1} / A2924 cm^{-1} (This absorbances ratio represents the change in the C=O of aldehyde group asymmetrical -C-H (CH_2) stretching vibration.) for the oils depending upon oil samples used. The decrease was referred to the decrease in the carbonyl groups which results from the breakdown of primary oxidative products and the hydrolysis of triglycerides because of heating.

From the IR result it can also be noticed that the intensity of the absorbance band at 1740 which represent the aldehyde group increases, this increase shows that there is an increase in the concentration of the aldehyde groups in the oil samples that used in frying food (sample 2 and sample 3) than the control group which according to the above finding results in the high level of the cytotoxicity observed.

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