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Effect of antioxidants on rats fed on thermally oxidized oil (fried oil)

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Abstract

The aim of the present study is to investigate the effect of different concentration of antioxidants on oxidative stress in rats fed on an oxidized oil. Sunflower oil was submitted to fryer treatments at temperature between (180-190 °C) on intermittent periods for 24 hrs. The changes in chemical properties of frying oil compared to fresh sunflower oil were investigated. Data illustrate the presence of an increase in acid value, peroxide value, polymers and a decrease in iodine number. Fatty acids constituents of this tested oil were also investigated. Fifty-four rats weighing approximately 100 grams each were divided into nine groups, each group containing six rats. Groups S2 and S3 fed on diets containing oxidized oil (15 %) and the other groups S4, S5, S6 S7, S8 and S9 fed on the same diet used in group S2 and supplemented with different concentration of antioxidants BHT (.02 gm/100gm oil), sodium selenite (1.5 mg / kg diet), β-carotene (10 mg/200gm rat/day), anthocyanin (1.43mg/200gm rat/day), cinnamon essential oil (0.9 gm/100 gm oil) and clove essential oil (0.9 gm/100 gm oil) respectively. Rats fed oxidized oil (S2 and S3) had higher concentrations of cholesterol, LDL-cholesterol and triglycerides. Although, the concentration of total thyroxine in plasma of rats fed oxidized diet (S2 and S3) was higher than rats fed the oxidized oil and supplemented with different concentrations of antioxidants. The damage caused by the oxidized oil was revealed by a significantly rise in lactic acid dehydrogenase activity (LDH) and thiobarbituric ácid reactive substances (TBARs) in plasma of rats in groups S2 and S3 Compared to other groups (S4, S5, S6 S7, S8 and S9). On the other hand, BHT as synthetic antioxidant at 200 ppm induced significant decrease in TBARs and increase in the LDH activity and function (liver and kidney). Microscopical examinations of thyroid gland , heart , liver and kidney tissues of rats treated with natural antioxidants had the histological characters as that of the control rats whilst, the treatment of BHT at 200 ppm altered the features of rat liver, kidney, thyroid gland and severely damaged rat heart tissues.

Introduction

In developed countries, Frying is a process currently used for the production of millions of tons of snacks worldwide. In fast food restaurant, fat is heated in fryers for up to 18 h daily, at temperatures close to 180°C. Fried foods are absorbing appreciable amount of oil reaching up to 40

% during frying ***(Houhoula et al., 2003)***. During the deep frying process, a series of degradation reactions, including autoxidation, thermal oxidation, polymerization and cyclization occur in the frying fat ***(Paul and Mittal 1997)***. The oxidation products in the used frying oils are commonly named as the polar compound fraction. Thermally oxidized fats contain a large number of lipid peroxidation products such as peroxides and hydroperoxides. The oxidized TG contain at least one oxygenated function in the esterfied fatty acids and may be a mixture of epoxide and ketone. The dimers are complex structure in which TG monomers are covalently linked through C-C or C-O-C bonds ***(Chao et al., 2001)***. Therefore, in many European countries rules that oil should be discarded when its total polar material content is higher than 25 %. The presence of lipid peroxides in food is undesirable because nutritional value decreases with the destruction of unsaturated fatty acids and other essential food constituent that possess an unsaturated lipid structure ***(Garrido-Polonio et al., 2004a)***.

The physiological effects of primary and secondary lipid peroxidation products are different. Primary lipid peroxidation products are highly toxic when administered parenterally but less toxic when given orally, probably due to low digestibility. Secondary lipid peroxidation products are particular physiological importance because they are highly digestible ***(Eder et al., 2002)***. Feeding thermally oxidized fat increases the concentrations of lipid peroxidation products in tissues, frying oil intake can cause tissue damage and may play a role in the inflammatory process of autoimmune disease ***(Hsieh and Lin, 2005)***. Oxidized fatty acids can be readily absorbed by the intestine, esterified into complex lipids and incorporated into lipoproteins ***(Penumetcha et al., 2000)***. Also, accumulating evidence suggests that oxidized fat and lipid oxidation products in the diet can also elevate levels of LDL cholesterol, triglyceride, and VLDL ***(Eder et al., 2003 and Brandsch et al., 2004)***. Plasma cholesterol, triglycerides and LDL-cholesterol above normal levels are closely related to the incidence of atherosclerosis and coronary artery disease ***(Tebib et al., 1997)***. Dietary oxidized fats could also enhance oxidative modification of LDL through their lipid peroxidation products and appears to affect several steps of atherogenic process ***(Santos et al., 2002)***.

Antioxidants are essential in preventing the cellular damage caused free radicals and free-radical-modified lipid peroxidation. These antioxidants probably exert their effects through their ability to scavenge reactive oxidants ***Ozdil et al., (2004)***. Recent studies have shown that essential oils such as cinnamon and clove, Anthocyanins, Carotenoids and selenium have powerful function as antioxidants and ability to scavenge reactive oxidants and have synergistic effect in the prevention of biological membranes from oxidants ***(Groff and Gropper, 2000; Burda and Oleszek, 2001; Suzuki and Ogra, 2002; Wu et al ., 2002*** *and* ***El-massary et al ., 2003)***.

The aim of the present study is to investigate the effect of different concentration of antioxidants (BHT, selenium, β-carotene, anthocyanins and cinnamon and clove essential oils) on

oxidative stress which increased by the oxidized oil and study their effects on serum lipid profile, liver and kidney function tests and thyroid gland hormones of rats received fried oil as well as the histopathological effect on liver , kidney , thyroid gland and heart tissues compared with nutraceutical tissues.

Materials and Methods

Materials

Local frying oil: Frying oil samples were received from several local fast food restaurants (frying local oil). In addition, all oil samples were collected from the fryer used to fry only potatoes products. All oil samples were kept at 4C until further analysis using chemical methods to determine the level of oil rancidity.

Sunflower fresh oil: was purchased from Sila Edible Oil Co., S.A.E, Koum Oshiem Fayoum.

Spices: the following spices, cinnamon (*Cinnamomum zeylancium*) and clove (*Syzygium aromaticum*) were purchased from Pharmaceutical Science Laboratory, National Research Centre, Giza, Egypt.

Butylated hydroxytoluene (BHT): as synthetic antioxidant (of purity 99.9%) was obtained from Naarden International Company.

Methods

Preparation of frying oil sample: Sample of sunflower oil was subjected to heat treatment comparable to that of deep – fat frying. A stainless steel laboratory fryer, sliced potatoes were put into a frying basket , containing refined sunflower oil at a temperature between (180-190°C).The oil intermittently frying accounted for 24 hrs. (3 hrs./day) ***Eder et al., (2003)*** and ***Kerolles (2003).***

Physicochemical variables: The acid value, peroxide value, iodine number, polymers and fatty acids were determined according to the methods described by ***A.O.A.C. (2000), Wu and Nawar (1986)*** and ***Aura et al., (1995).*** Thiobarbituric acid (TBA) value was determined according to the method described by ***Ottolenghi (1959).***

Extraction of essential oils from spices: The essential oils of cinnamon and clove fruits were obtained by water distillation in a glass apparatus, for 4 hours. The separated volatile oil was dried over anhydrous sodium sulphate before holding glass bottles at -20°C, according to ***Guenther (1961)***

Kits (Total cholesterol , High density lipoprotein cholesterol, Low density lipoprotein cholesterol, Triglycerides , Total lipids, Alanine amino transferase (ALT), Aspartate amino

transferase (AST) , Alkaline phosphatase (AP) ,Total bilirubin ,γ-L-Y-Glutamyl transferase (γ -GT) ,Creatinine , Urea and Uric acid :were obtained from Randox Laboratories Ltd., Diamond Road, Crumlin , Co., Antrim, United Kingdom,BT294QY. Kits Tetraiodothyronine (T_4) and triiodothyronine (T_3) were obtained from COAT-A-COUNT CO., USA.

Serum total cholesterol (TC.), high density lipoprotein (HDL), low density lipoprotein (LDL), VLDL- cholesterol and triglycerides (TG.) were determined by using the methods described by ***Richmond (1973), Assmann (1979), Wieland and Seidel (1983), Wallach (1992) and Fossati and Prencipe (1982),*** respectively.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed by the method of ***Bergmeyer and Harder (1986).*** Alkaline phosphatase (AP) activity was measured at 405 nm by the formation of paranitrophenol from para-nitrophenylphosphate as a substrate using the method of ***Varley et al., (1980).*** Serum total bilirubin was measured using the method of ***Walters and Gerade (1970).***γ- L-Y-Glutamyl transferase (γ -GT) was measured using the method of ***Szasz (1969).*** Lactic acid dehydrogenase activity was determined by enzymatic colorimetric method according to ***Rec (1972).***

Creatinine was measured using the method of ***Henry (1974),*** Urea was measured using the method of ***Fawcett and Scott (1960)*** while Uric acid was measured using the method of ***Caraway (1955).***Tetraiodothyronine (T_4) and triiodothyronine (T_3) concentrations were determined according to ***Hollander et al., (1974)*** by radioimmunoassay (RIA).Thiobarbituric acid reactive substances was determined by the colorimetric method described by ***Meltzer et al., (1997).***

Histopathological examination :Sample from the liver, kidneys, heart and thyroid gland were collected from rats in all groups at the end of experiments (60 days), fixed in 10% neutral buffered formalin, dehydrated in alcohol, cleared in xylol and embedded in paraffin. 4μ thick sections were prepared and stained with Hematoxylene and Eosin ***(Carleton, 1976).***

Diet:

The composition of the basal diet (g/kg): casein 217; corn starch, 583; fresh vegetable oil 150; mineral mix. , 40; vitamin mix. , 10 ***(Zamore et al ., 1991 , Kerolles, 2003 ;Hegested et al.,1941 and Campiell,1961*** , respectively) .

Design :

A total of 54 young male Albino rats, average weight of 100 g. raised in the animal house of the Ophthalmology Research Institute, Giza, Egypt, were used in the present study. The animals were equally divided into 9 groups (each of 6 rats) according to the type of oil as follows:

1- Six rats were fed on a basal diet (S1).

2- Six rats were fed on a basal diet except that the fresh sunflower oil portion was replaced by 15 % of laboratory frying oil (S2).

3- Six rats were fed on a basal diet except that the fresh sunflower oil portion was replaced with 15 % of restaurant frying oil (S3).

4- Thirty six rats were divided into 6 groups. Each of 6 rats were fed on a basal diet and the fresh sunflower oil portion was replaced with 15 % of laboratory frying oil (S4, S5, S6, S7, S8 and S9). [S4 the 4th group was receiving BHT (.02 gm/100gm oil), ***Badee et al ., 2005***], [(S5) ,the 5th group was receiving selenium as sodium selenite (1.5 mg / kg diet), ***Ahmed, 2004]*** , [S6 the 6th group was receiving β-carotene (10 mg/200gm rat/day), ***El-dkak, 2004***], [(S7), the 7th group was receiving , anthocyanin (1.43mg/200gm rat/day), ***El-Dakak, 2004***],[(S8), the 8th group was receiving cinnamon essential oil (0.9 gm/100 gm oil) and (S9), the 9th group was receiving clove essential oil (0.9 gm/100 gm oil), ***Badee et al ., 2005*** .

Blood sampling:

Blood samples were taken at the end of the experiment being 60 days of the administration of the tested materials (BHT, selenium, β-carotene, anthocyanin and (cinnamon and clove) essential oils). The blood samples were obtained from orbital venous plexus by means of fine capillary glass tubes according to the method described by ***Schermer, (1967).***

Statistical analysis:

The obtained results were subjected to statistical analysis using the standard analysis of variance as outlined by ***Snedecor and Cochran (1980).*** Factorial analysis containing two factors in completely randomized design as described by ***Gomez and Gomez (1984)*** was also used. The difference between means were tested for significance against the least significant range (LSR).

Results and discussion

The results demonstrate the occurrence of high increase in the acid value, peroxide value and thiobarbituric acid value of the fried sunflower oil (laboratory and restaurant) compared to fresh oil. Meanwhile, iodine values of fried sunflower oil were decreased by prolonging the fried process (Table 1).

The initial polymer content of fresh sunflower oil was 0.27 % and this value was increased in restaurant and laboratory frying oil as shown in Table (1). ***Sanchez-Muniz and Bastida (2003)*** *and* ***Romero et al ., (2006)*** have indicated that oils with a low linoleic acid and a high oleic acid content tend to be oxidized more easily than oils with a higher linoleic acid and lower oleic acid content which have a greater tendency to undergo polymerization. This fact is of nutritional relevance due to polymers, which are potentially toxic. These compounds are more actively

digested and absorbed *(Lopez – Varela et al., 1995; Marquez – Ruiz and Dobarganes, 1996* and *Gonzalez –Munoz et al., 1998, 2003).*

The fatty acid composition of the used oils across 8 days of frying is presented in Table (1). The results show generally that in all frying oil , there were decreases in linoleic acid (18:2) and oleic acid (18:1) ,whereas myristic acid (14:0), palmitic acid (16:0) and stearic acid (18:0) slightly increased at the end of frying time. In particular, during frying, PUFA decreased and total SFA increased. ***Orthoefer and Cooper (1996) , Arroyo et al.,1995*** *and* ***Jorge et al.,1997*** have pointed out that heat treatment of fats induces modifications of fatty acids with two or three double bonds. In the current study the level of PUFA tended to decrease, whereas that of SFA increased.

Also, the results show that the decrease in C18:2 and C 18:1 and the increase in C14:0, C16:0 and C18:0 across 8 consecutive days of frying were higher in the (laboratory and restaurant) oil compared to sunflower fresh oil. On the other hand, ***Ramadan et al., (2006)*** found that the ratio of linoleic acid to palmitic acid tended to decrease at a diminishing rate by increasing the frying period. In the sunflower oil, the drop in the C18:2 /C16:0 ratio was lower than that of the restaurant oil.

Lipid profile:

Table (2) shows the concentrations of serum total cholesterol (TC) and low density lipoprotein (LDL-C) of rats .The concentration of (TC) and (LDL-C) in serum were significantly influenced by the dietary oxidized fats in all groups. Rats fed on the oxidized fat fried (S2 and S3) had higher concentrations of (TC) in serum than those fed on fresh fat and all groups. Rats fed on diets with anthocyanins or (cinnamon and clove) essential oils and BHT at 200ppm had lower concentrations of (TC) and (LDL-C) in serum than those fed on diets with selenium and β-carotene. Also, no significant change was observed between natural and synthetic antioxidant in all groups. These results agree with that ***Eder and Stangl (2000)*** and ***Garrido-Polonio et al., (2004 b)*** who found that linoleic acid is hypocholesterolaemic. A high intake of linoleic acid has been shown to decrease LDL-cholesterol and cholesterol. Thus, one explanation for the rise of serum total–cholesterol and LDL-cholesterol, might be due to lower intake of linoleic acid in dietary oxidized oil compared with fresh oil. Dietary oxidized oils increase oxidative stress and the presence of oxidized LDL-cholesterol and other lipoproteins. Oxidation converts LDL-cholesterol to a form that is rapidly taken up and degraded by macrophages and increased degradation of unoxidized LDL-cholesterol. Antioxidants are inhibiting metabolism of LDL-cholesterol and reduce toxicity of oxidized LDL-cholesterol ***(Schwenke, 1998).*** Other explanation by ***Khan- Merchant et al., (2002)*** is the possible mechanism for the action of oxidized fatty acids based on the observation that 13- hydroxylinoleic acid (13- HODE) and oxidized fatty acids may act as bile salt enhancers and increase the solubilization and absorption of cholesterol , leading to high plasma cholesterol levels. Oxidized

lipoproteins play an important role in the development of atherosclerosis *(**Steinberg et al., 1989** and **Witztum and Steinberg 1991**).*

Serum high density lipoprotein cholesterol (HDL - C): as shown in Table (2) indicated that the rats fed on natural and synthetic (BHT) antioxidant groups showed a slight decrease in the concentrations of (HDL -C) compared with the control rats (fresh oil). Meanwhile, fried oil groups (S1 and S2) had high decrease in the concentrations of (HDL - C) compared to all groups. ***Yokozowa et al., (2002)*** found that antioxidant rich foods increased the response to the oxidative damage in the pathogenesis of many diseases and increased HDL-C.

Serum triglycerides (TG) and very low density lipoprotein cholesterol (VLDL – C): The concentration of (TG) and (VLDL – C) were studied, as shown in table (2). These data indicate that, in rats fed on diet containing oxidized oil (S2 and S3), the concentration of (TG) and (VLDL – C) increased significantly from (90.47 mg/dl (S1) to 179.2 mg/dl and 187.5 mg/dl) and (18.09 mg/dl (S1) to 35.84 mg/dl and 37.50 mg/dl), respectively in groups S2 and S3. Meanwhile, the other groups (S3, S4, S5, S6, S7, S8 and S9) fed on diet containing oxidized fat and supplemented with different concentration of antioxidants had lower value compared with groups S2 and S3. These results are in agreement with ***Naruszewicz et al., (1987)*** and ***Brandsch et al., (2004)*** who observed that, there is a 10- fold increase in the uptake of chylomicrons after consumption of heated oil. There is some evidence that highly oxidized oils accelerate the development of atherosclerosis ***(Strapans et al., 1996)***, and some studies report that increased VLDL-C and chylomicrons of rats, rabbits and humans following ingestion of oxidized oils ***(Hayam et al., 1995, Strapans et al., 1994, 1996).***

Liver function:

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP) , γ –GT and total bilirubin activities : The data in Table (3) show the activities of ALT, AST , AP , γ –GT and total bilirubin for control rats (fresh oil) and the values were slightly increased during the whole experiment (60 days) . The administration of two groups of fried oil (S2 and S3) and BHT at 200ppm to experimental rats induced significant increases in serum ALT, AST and AP activities at the end of experiment. On the contrary, no significant differences occurred among all natural antioxidants. ***Baron (1987)*** mentioned that the rise in the activities of ALT, AST and AP in rat serum is a sign of hepatocelluor damage. This case had been found in rats administered BHT and fried oils without natural antioxidants. These results appear to be in agreement with those of ***Farag et al., (2006)***. The outstanding augmentation that was observed in serum ALT and AST may be a result in formation of free radicals in the oils that were subjected to a severe heating ***(Nawar, 1979)***. These compounds produced from lipid peroxidation were suggested to one of the major factors involved in liver injury as reported by ***Slater (1978)*** and ***Recknagel (1983).*** They also

showed that peroxidative breakdown of membrane lipids certainly occurred following the formation of free radicals that attack the linkage of unsaturated fatty acids. The short – chain compounds were produced due to heating of vegetable oils such allyl alcohol exhibited periportal necrosis ***(Rees and Torlow, 1979).***

Serum LDH activity is presented in Table (3). From the obtained results, a significant increase of serum LDH activity in groups fed on diet containing oxidized oil (S2 and S3) when compared with the normal group (S1). In our study, it was observed that, enzymes such as LDH was released into the blood. The increase in the serum activity of this enzyme was directly proportional to the degree of cellular damage and is an indicator of liver destruction ***Ozdil et al., (2004).*** Also, a significant decrease was noted in the groups fed on diet containing oxidized oil and supplemented with different concentration of antioxidant (S5, S6, S7, S8 and S9) by (48.16 ,64.38 ,41, 58.76 and 42.25 %) respectively, when compared with S2 group. In contrast, the highest value of LDH was observed in treatment 4 (S4) when compared with the control group (S2) or all treatments (S5, S6, S7, S8 and S9). In this concern ***Sulzle et al., (2004)*** reported that, many of the effects caused by oxidized fats are mediated by oxidative stress. Dietary oxidized fat may be increased as the result of formation of reactive oxygen species (ROS) and may induce increased damage proteins in the liver through enhanced generation of ROS and increased lipid peroxidation of the cell membrane. This leads to increased membrane fluidity, disturbances of calcium homeostasis, and finally cell death.

Kidney function:

Serum uric, urea and creatinine (kidney functions) as shown in the Table (3), the fried oil rats (laboratory and restaurant oxidized oil) and BHT at 200ppm showed significant and gradual increases in the concentrations of uric, urea and creatinine during the entire experiment. In contrast, anthocyanin, essential oils, selenium and β-carotene rat groups indicate that there were no significant changes of serum uric, urea and creatinine. These results are in agreement with ***Kerolles (2003)*** *and* ***Farag et al., (2006).***

Thyroid hormone concentrations:

Table (4) shows plasma tetraiodothyronine (T_4) and triiodothyronine (T_3) levels of rats fed on diet containing oxidized oil (15 %) or the rats fed on the same diet supplemented with different concentrations of antioxidants. Rats fed on the oxidized oil (S2 and S3) had greater concentrations of total T_4 in serum 123.42 and 137.15 nmol/L respectively, than that obtained in rats fed on fresh oil (S1) .Also, on supplementing the diet with different concentration of antioxidants (S4, S5, S6, S7, S8 and S9) significantly increased total thyroxine when compared with normal group (S1). However, the rate of increase in groups S2 and S3 was higher compared to all groups. The concentration of T_3 did not differ among the nine groups. Hence, rats fed on the oxidized oil had a higher ratio of T_4

and T_3 than rats fed on fresh oil. The same result was found by ***Eder et al., (2002)*** who found that, marked increase in the concentrations of free and total thyroxine. Other study by ***Liu and Huang (1995)*** found that feeding oxidized oils reduced the activity of glutathione peroxidase and reduced deiodination of thyroxine to triiodothyronine by 5'IDI enzyme (type 1, iodothyronine 5' deiodinase) ***Eder, (1999).***

Malondialdehyde concentration:

Malondialdehyde concentration in serum: The concentration of malondialdehyde was studied, as shown in table (5). These data indicate that, in rats fed on diet containing oxidized oil (S2 and S3), the concentration of malondialdehyde level was increased significantly from 0.55 nmol/L (S1) to 3.89 nmol/L and and 4.36 nmol/L in groups S2 and S3 respectively. On the other hand, the other groups (S4, S5, S6, S7, S8 and S9) which fed on diet containing oxidized fat and supplemented with different concentration of antioxidants had lower value when compared with groups S2 and S3.The present results agreed with those data presented by ***Garrido-Polonio et al., (2004b)*** who found that, the rats fed used sunflower-seed oil had higher TBARs contents in serum and in all lipoproteins than rats fed unused sunflower-seed oil. Also, ***Izaki et al., (1984)*** reported that liver-TBARs was increased significantly in rats receiving four levels of thermally oxidized oils in comparison with their counterparts fed fresh oils.

Histopathological examination:

The influence of BHT (200ppm) and natural antioxidants (selenium, β carotene, anthocyanins, cinnamon essential oil and clove essential oil) on liver, kidney, thyroid gland and heart tissues of male albino rats was microscopically examined.

Histopathological examination: Figure (1) shows the microscopic examination of liver, kidney tissues of rats fed on a normal diet (S1).The microscopic examination of liver tissues group (S1) as certain the presence of normal histological structure of hepatic parenchyma, including normal hepatic cords, central veins and portal areas. The microscopic examination of kidney tissues group (S1) demonstrated the occurrence of normal cortical and medullary structures including renal tubules and glomeruli.

Microscopical examination of liver and kidney tissues of rats fed on natural antioxidants: the hepatic tissues showed normal hepatocytes, central veins and portal area. Also, the kidney parenchyma of rats that ingested natural antioxidants appeared to be normal either in cortex or medulla control rats.

Microscopical examination of liver tissues of rats fed on a fried oil: The liver of rats from group S2 showed vacuolation and dissociation of hepatocytes group (S2). Moreover, liver of

rats from group S3 showed dissociation of hepatocytes, portal edema and few leucocytic cells infiltration in the portal area group (S3).

Microscopical examination of kidney tissues of rats fed on fried oil: The kidney of rats from group S2 showed no changes except for congestion of renal blood vessels group (S2). However, kidneys of rats from group S3 showed dilatation and congestion of renal blood vessel, atrophy of glomerular tuft and distension of Bowman s space group (S3)

Microscopical examination of liver and kidney tissues of rats given butylated hydroxyl toluene (BHT): Figure (1) elucidates the microscopic examination of liver tissues of rats administered BHT (200ppm) at the end of the experiment. The microscopic examination for liver tissues showed marked pathological changes such as focal hemorrhage dispersed the hepatocytes, and hyperplasia of epithelial lining bile duct associated with portal edema group (S4)

The microscopic examination of kidney tissues (Figure 1) of rats given BHT (200ppm) revealed granularity of the epithelial lining of the renal tubules , pyknosis of their nuclei and atrophy of glomerular tuft group (S4) as well as necrosis of renal tubules and hemorrhage were also noticed in examined cases .

On the other hand, the microscopic examination of thyroid gland and heart tissues (Figure 2) from control rats (S1) described no histopathological changes In other words, the natural antioxidants did not cause any adverse effect on thyroid gland and heart tissues. Meanwhile, microscopical examination of thyroid gland and heart tissues of rats fed on a fried oil group S2 showed distension of the thyroid follicles with deeply eosinphilic colloid group (S2) and lined with flattend epithelium. However, thyroid tissues of rats from group S3 revealed cystic dilatation of thyroid gland follicles .

Microscopical examination of heart tissues of rats from group (S2) revealed zanker's necrosis of muscle fibers associated with slight inter muscular edema. However, heart tissues of rats from group (S3) showed vacuolation of blood vessel wall and granularity of cardiac muscle fibers.

Microscopical examination of thyroid gland and heart tissues of rats given (BHT): Figure (2) elucidates the microscopic examination of thyroid tissues of rats given BHT (200ppm) at the end of the experiment. The microscopic examination for thyroid gland tissues showed hyperplasia of follicular cells associated with flattened follicular cells lining the macrofollicles (group S4)

The histopathological examination of heart tissues (Figure 2) of rats given BHT (200ppm) showed vacuolation of some cardiac muscle fibers associated with inter muscular edema and hemorrhage (group S4).

Radical scavengers by essential oils (**Fuhrman *et al* .,2000; Badei *et al.*, (2002); Wu *et al* .,2002** and **El-massary *et al* ., 2003**) . and other natural sources such as anthocyanins, carotenoids and selenium play an important role in the prevention of coronary heart diseases **(Dreosti , 1996; Groff and Gropper, 2000** and **Badee *et al* ., (2005)** , hypercholesterolaemia **(Itaya and Igarash , 1992** and **Farag *et al* ., 2006)** , possess anti – allergic , anti – inflammatory , anti – viral , anti – carcinogenic , anti – proliferative properties **(Manach *et al* . , 1996)** and powerful antioxidant substances **(Farag *et al* ., 2002 , 2003) .**

Consequently, one would suggest feeding natural antioxidants to decrease any deleterious effect on human health caused by frying oil. In general, the data for histopathological examinations are in accordance with results of biochemical measurements of serum rat liver, kidney function tests and thyroid gland hormones.

Table (1): Characteristics and fatty acid composition of the fresh sunflower oil and oxidized frying oil.

Treatments	Sunflower oil (fresh)	Oxidized oil	
		Sunflower oil (laboratory)	oil (restaurant)
Acid value (mgKOH/g oil)	0.04	0.98	1.72
Peroxide value (meq.peroxides/kg oil)	0.42	40.14	87.81
Iodine value (gI_2/100glipids)	133.20	121.04	107.82
Thiobarbituric acid mmol / kg	0.07	19.86	27.95
Polymer (%)	0.27	2.50	3.72
Fatty acids			
C14:0	-	5.68	8.11
C16:0	10.09	14.12	17.26
C18:0	5.23	9.72	14.82
C18:1	17.67	14.00	10.94
C18:2	67.01	56.48	48.87
US	84.68	70.48	40.19
TS	15.32	29.52	59.81
U/S	5.52	2.38	0.67

Table (2): Effect of different concentration of antioxidants on serum total-cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol and triglycerides in rats fed on oxidized fat for 60 days.

Treatment	T.cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Triglycerides (mg/dl)
S1	89.04 ± 0.86 ^{c}	42.83 ± 0.17 ^{a}	27.79 ±0.92 ^{d}	18.09 ± 0.16 ^{c}	90.47 ± 0.80 ^{c}
S2	139.30 ± 9.93 ^{ab}	29.23 ± 0.34 ^{c}	73.87 ± 9.32 ^{b}	35.84 ± 0.87 ^{a}	179.20 ± 4.34 ^{a}
S3	150.10 ± 5.83 ^{a}	26.80 ± 0.37 ^{d}	85.43 ±6.53 ^{a}	37.50 ± 1.20 ^{a}	187.50 ± 5.98 ^{a}
S4	117.80 ± 2.13 ^{b}	36.74 ± 0.34 ^{b}	55.79±2.42 ^{c}	24.91 ± 1.20 ^{b}	124.60 ± 5.98 ^{b}
S5	119.0 ± 13.04 ^{b}	36.95 ± 0.02 ^{b}	56.56±12.30 ^{c}	25.17 ± 0.89 ^{b}	125.90 ± 4.43 ^{b}
S6	119.4 ± 13.80 ^{b}	36.61 ± 0.37 ^{b}	57.82±14.27 ^{c}	25.37 ± 0.12 ^{b}	126.90 ± 0.60 ^{b}
S7	117.70 ± 6.40 ^{b}	36.97 ± 0.22 ^{b}	55.34±7.09 ^{c}	24.90 ± 0.55 ^{b}	124.50 ± 2.75 ^{b}
S8	118.3 ± 14.19 ^{b}	36.44 ± 0.34 ^{b}	56.34 ±14.53 ^{c}	25.00 ± 0.68 ^{b}	125.0 ± 3.41 ^{b}
S9	117.10 ± 4.01 ^{bc}	37.18 ± 0.38 ^{b}	54.91 ± 4.87 ^{c}	24.61 ± 0.50 ^{b}	123.10 ± 2.47 ^{b}
LSD	28.22	0.80	9.49	2.13	10.66

Each value represents the mean ± SE.

The mean values with different superscript alphabets indicate significant differences ($P<0.05$) using LSD test.

Table (3): Effect of different concentration of antioxidants on serum Liver function (ALT, AST, AP, γGT, total-bilirubin and LDH activity) and kidney function (creatinine, urea and uric acid) in rats fed an oxidized fat oil for 60 days.

Treatment	Liver Function						Kidney Function		
	ALT (U/L)	AST (U/L)	AP (IU/L)	γGT (U/L)	T.bilirubin (mg/dl)	LDH (U/L)	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
S1	21.29±1.41^{d}	27.93±1.06^{d}	62.05±0.78^{d}	3.70±0.25^{d}	1.01±0.01^{e}	101.41± 4.51^{e}	0.39±0.02^{d}	22.19±1.08^{d}	3.10±0.05^{d}
S2	30.24±2.0^{bc}	41.21±2.91^{bc}	84.75±1.98^{b}	6.03±0.03^{ab}	1.38±0.01^{a}	457.16± 5.63^{b}	0.74±0.07^{bc}	33.82±2.62^{a}	3.91±0.07^{bc}
S3	34.84±1.70^{ab}	49.47±0.38^{ab}	91.82±2.92^{a}	6.55±0.50^{a}	1.40±0.01^{a}	644.37± 4.58^{a}	0.75±0.03^{b}	32.31±2.20^{ab}	4.75±0.39^{a}
S4	39.73±1.08^{a}	56.80±1.18^{a}	93.23±1.53^{a}	5.45±0.12^{b}	1.43±0.02^{a}	584.36± 9.56^{a}	1.32±0.17^{a}	35.87±0.84^{a}	4.53±0.28^{ab}
S5	28.69±1.94^{c}	37.79±3.27^{c}	74.44±1.05^{c}	4.21±0.21^{cd}	1.15±0.01^{b}	236.98± 3.19^{cd}	0.54±0.03^{d}	29.18±0.39^{bc}	3.61±0.32^{cd}
S6	28.83±1.89^{c}	39.85±3.97^{c}	73.81±1.85^{c}	4.32±0.11^{cd}	1.11±0.01^{b}	162.82± 1.98^{de}	0.50±0.03^{d}	28.47±0.75^{bc}	3.67±0.33^{cd}
S7	27.41±1.89^{c}	38.13±4.73^{c}	73.61±1.95^{c}	4.44±0.02^{c}	1.12±0.01^{b}	269.62± 5.20^{c}	0.54±0.02^{d}	27.43±0.76^{c}	3.55±0.30^{cd}
S8	28.40±1.74^{c}	39.24±4.42^{c}	71.81±1.09^{c}	4.24±0.2^{cd}	1.11±0.01^{b}	188.54± 3.3^{cd}	0.53±0.03^{d}	28.03±1.90^{bc}	3.59±0.18^{cd}
S9	26.87±2.08^{cd}	36.40±2.15^{cd}	71.28±1.13^{c}	4.11±0.11^{cd}	1.14±0.04^{b}	264.01± 1.48^{c}	0.56±0.02^{cd}	27.96±0.63^{bc}	3.52±0.17^{cd}
LSD	5.01	9.19	3.65	0.65	0.05	78	0.19	4.44	0.67

Each value represents the mean ± SE. The mean values with different superscript alphabets indicate significant differences (P<0.05)using LSD test

Table (4): Effect of different concentration of antioxidants on thyroid hormones concentrations in rats fed on oxidized fat for 60 days.

Treatments	T3 (nmol/L)	T4 (nmol/L)	T3 / T4
S1	1.38 ± 0.03 ^{a}	84.10 ± 0.30 ^{f}	61.18 ± 0.89 ^{d}
S2	1.34 ± 0.03^{a}	123.42 ± 3^{b}	92.52 ± 3.98^{b}
S3	1.32 ± 0.02^{a}	137.15 ± 3.04^{a}	103.96 ± 3.88^{a}
S4	1.31 ± 0.02^{a}	94.5 ± 1de	72.42 ± 0.07^{c}
S5	1.37 ± 0.02^{a}	99.08 ± 1.45^{cde}	72.58 ± 0.26^{c}
S6	1.37 ± 0.02^{a}	107.65 ± 2.5^{c}	78.62 ± 2.97^{c}
S7	1.35 ± 0.05^{a}	102.56 ± 2.56^{cd}	76.27 ± 0.65^{c}
S8	1.36 ± 0.04^{a}	92.16 ± 3.05^{ef}	69.14 ± 4.1^{cd}
S9	1.38 ± 0.03^{a}	94 ± 4.89^{de}	68.23 ± 5.03^{cd}
LSD	0.09	8.76	9.72

Each value represents the mean ± SE.

The mean values with different superscript alphabets indicate significant differences (P<0.05) using LSD test.

Table (5): Effect of different concentration of antioxidants on malondialdehyde Concentration in serum of rats fed an oxidized fat for 60 days.

Treatment	TBARS (nmol/L)
S1	0.55 ± 0.05 ^{e}
S2	3.89 ± 0.58^{a}
S3	4.36 ± 0.15^{a}
S4	2.39 ± 0.19^{b}
S5	1.2 ± 0.1^{de}
S6	1.48 ± 0.37^{cd}
S7	2.39 ± 0.1^{b}
S8	2.54 ± 0.04^{b}
S9	2.06 ± 0.1^{bc}
LSD	0.79

Each value represents the mean ± SE.

The mean values with different superscript alphabets indicate significant differences (P<0.05) using LSD test.

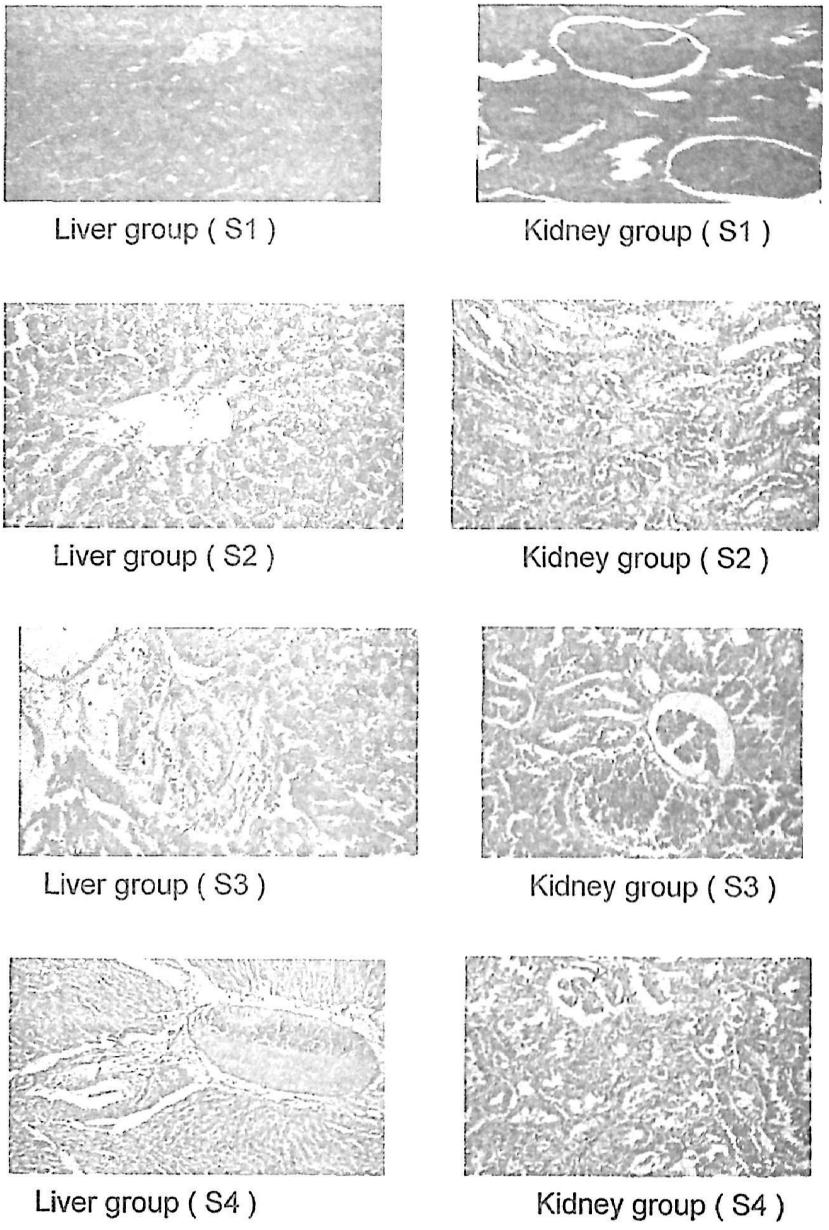

Figure (1): *Histopathological examination of liver and kidney tissues of rats fed only basal diet , oxidized oil and BHT (200 ppm).*

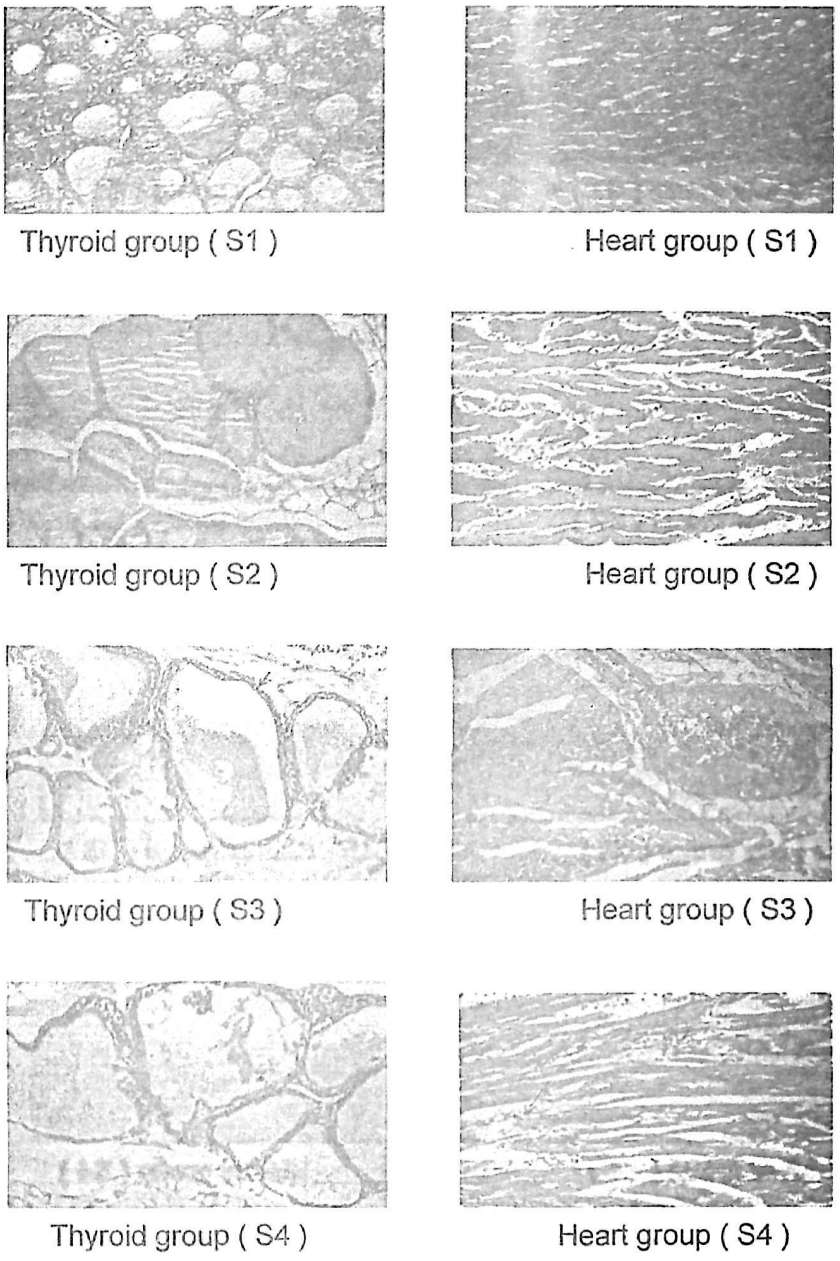

Figure (2): Histopathological examination of thyroid glands and heart tissues of rats fed only basal diet , oxidized oil and BHT (200 ppm).

References

Ahmed, M. H. M. (2004).
Biochemical studies on the relation between thyroxin hormone and selenium. Ph. D. Thesis, Fac. Of Agri., Biochemistry Dep., Cairo Univ., Egypt

A.O.A.C. (2000).
Official Methods of Analysis of the Association of Official Analytical Chemists, 17th Ed, edited by W. Horwitz, A.O.A.C., Washington, DC.

Arroyo, R., Cuesta, c., Sanchez- Montero, J. M. and Sanchez–Muniz, F. J. (1995).
High performance size exclusion chromatography of palm olein used for frying. Fat Sci. Technol., 97: 292-296.

Assmann, G. (1979).
A fully enzymatic colorimetric determination of HDL-cholesterol in the serum. Internist, 20: 559.

Aura, A. M.; Forssell, P.; Mustranta, A. and Poutanen, K. (1995).
Transesterification of soy lecithin by lipase and phosphilipase . J. Am. Oil Chem. Soci., 72: (11): 1375-1379.

Badee, Adel, Z.M.; El-Akel, Ahmed, T.M.; Hafez, Saep, A. M.; Kawkab, A. Ahmed and Naglaa, H. M. Hassanen (2005).
Biochemical and histopathological effects of cinnamon , clove and ginger powders and their essential oils. The Third International Conference for Food Science and Technology, "Modernizing Food Industries", Feb. 22-24th, Cairo, Egypt,125-144.

Badei, A. Z. M.; El-Akel, A. T. M.; Faheid, S. M. M. and Mohmoud, B. S. M. (2002).
Application of some spices in flavoring and preservation of cookies. I. Antioxidant properties of cardamom, cinnamon and clove .Deutsche lebensmittel – Rundschua, 98 (5): 176-183.

Baron, D. N. (1987).
A Short Textbook of Chemical Pathology. 4 th edn . pp. 188-228. Kent, Great Britain: English Language Book Society Hodder and Stoghton Ltd.

Bergmeyer, H. U. and Harder, M. (1986).
A colorimeteric methods of determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Clin. Biochem., 24: 28 – 34.

Brandsch, C., Nass, N. and Eder, K. (2004).
A thermally oxidized dietary oil does not lower the activities of lipogenic enzymes in mammary glands of lactating rats but reduces the milk triglyceride concentration. J. Nutr., 134:631 – 636.

Burda, C. W. and Oleszek (2001).
Antioxidant and antiradical activities of flavonoids. J. Agric. Food Chem., 49: 2774-2779.

Campiell, T. A. (1961).Methodology of protein evaluation. RAG.Nutr,Document R.101 adds. 37, June Melting , New York.

Caraway, W. T. (1955).
Determination of uric acid in serum by a carbonate method. American Journal of Clinical Pathology 25: 840-845.

Carleton, H. (1976).
Carleton's Histopathological Technique. 4 thED. London, Oxford University Press, New York, Toronto.

Chao, P-M., Chao, C-Y., Lin, F-J and Huang, C-J. (2001).
Oxidized frying oil up-regulates hepatic acyl-COA oxidase and cytochrome P_{450} 4 A1 genes in rats and activates PPARα^1.J. Nutr., 131: 3166-3174.

Dreosti, I. E. (1996).
Bioactive ingredients: antioxidants and polyphenols in tea. Nutr. Rev., 54: 551-558.

Eder, K. (1999).
The effects of a dietary oxidized oil on the lipid metabolism in rats. Lipids, 34: 717-725.

Eder, K., Keller, U., Hirch, F. and Brandsch, C. (2003).
Thermally oxidized dietary fats increase the susceptibility of rat LDL to lipid peroxidation but not their uptake by macrophages. J. Nutr., 133: 2830-2837.

Eder, K., Skufca, P. and Brandsch, C. (2002).
Thermally oxidized dietary fats increase plasma thyroxine concentrations in rats irrespective of the vitamin E and selenium supply. J. Nutr., 132:1275-1281.

Eder, K. and Stangl, G. I. (2000).
Plasma thyroxine and cholesterol concentrations of miniature pigs are influenced by thermally oxidized dietary lipids. J. Nutr., 116 : 116-121.

El-Dkak, A, M.N. H. (2004).
Effect of natural antioxidants in grapes and carrots on cholesterol level. Ph. D. Thesis, Fac. Of Agric., Food Science and Technology Dep., Cairo Univ., Egypt.

El- Massry, Kh. F. (2003).
Inhibitory effects of some essential oils on cu^{2+} induced oxidative modification of human low density lipoproteins. Bull. Fac. Agric., Cairo Univ., 54 (1): 77-92.

Farag, R.S., Abd El–moien, N. M. and Mahmoud, E. A. (2002).
Antioxidant properties of olive phenolic compound on sunflower oil stability. Adv. Food Sci., 24, 99-105.

Farag, R. S., El-Baroty, G. S. and Amany, M. Basuny (2003).
The influence of phenolic extracts obtained from the olive plants (cvs. picual and kronakii) on the stability of sunflower oil. Intr. J. Food Sci. Techn., 38: 81-87.

Farag, R. S., Ebtesam A. Mahmoud, Amany M. Basuny and Rehab F. M. Ali (2006).
Influence of crude olive leaf juice on rat liver and kidney functions. International Journal of Food Science and Technology, 41: 790-798.

Fawcett, J.K. and Scott, J.E.(1960).
Enzymatic colorimetric method for determination urea in serum, plasma and urine. J. Clin. Path., 13,156-162.

Fossati, P. and Prencipe, L. (1982).
The determination of triglycerdes using enzymatic methods. Clin. Chem., 28: 2077.

Fuhrman, B.; Rosenblat, M.; Hayek, T.; Coleman, R. and Aviram, M. (2000).
Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation and attenuates development of atherosclerosis in atherosclerotic, Apolipoprotein E-deficient mice. J. Nutr., 130: 11424-1131.

Garrido-Polonio, C., Garcia-Linares, M. C., Garcia–Arias, M. T., Lopez–Varela, S., Garcia–Fernandez, M. C., Terpstra, A. H. M. and Sanchez– Muniz, F. J. (2004 a).
Thermally oxidized sunflower–seed oil increases liver and serum peroxidation and modifies lipoprotein composition in rats . British Journal of Nutrition , 42: 257-265.

Garrido-Polonio, C., Garcia-Linares, M. C., Garcia-Aias, M. T., Lopez-Varela, S., Garcia-Fernandez, M. C., Terpstra, A. H. M. and Sanchez-Muniz, F. J. (2004 b).
Thermally oxidized modifies lipoprotein composition in rats. British Journal of Nutrition, 92: 257-265.

Gomez, K. A. and Gomez, A. A. (1984).
'Statistical procedures for agriculture research" Book. PP. 207. John Wiley & Sons, Inc., New York.

Gonzalez-Munoz , M. J., Bastida , S. , Sanchez–Muniz , F. J., (1998).
Short term *in vivo* digestibility of triglyceride polymers, dimmers and monomers of thermoxidized palm olein used in deep–frying. Journal of Agriculture and Food Chemistry, 46: 5158-5193.

Gonzalez-Munoz, M. J., Bastida, S., Sanchez–Muniz, F. J. (2003).
Short term in vivo digestibility assessment of a highly oxidized and polymerized sunflower oil. Journal of the Science of Food and Agriculture 83: 413-418.

Groff, J., L. and Gropper, S. S. (2000).
Advanced nutrition and human metabolism. Wadsworth, a division of Thomson Learing, United States of American. Third Edition, PP. 440- 446 and 370.

Guenther, M. (1961).
"The essential oils."Vol. III, IV, 4th Ed..D. Van Nostrand Company, Inc. Princeton, New Jersy. Tornto, New York, London.

Hayam, I., Cogan, U. and Mokady, S. (1995).
Dietary oxidized oil and the activity of antioxidant enzyemes and lipoprotein peroxidation in rats. Nutr. Res., 15:1037-1044.

Hegested, D. M.; Mills, R. C.; Elvehijem, C. A. and Hart, F.B. (1941).
Choline in the nutrition of check . J.Biol.Chem.,138:459. ***Henry, R. J. (1974).*** Clinical Chemistry, Principles and Tecnics , 2 nd Edition, Harper and Raw, p. 525.

Hollander, C. S., Nihei, N., Burday, S.Z., Mitsuma, T., Shenkman, L. and Blum, M. (1974).
Clinical and laboratory observations in cases of triiodothyronine toxicosis confirmed by radioimmunoassay. Lancet, 609-611.

Houhoula, D., Oreopoulou, V. and Tzia, C. (2003).
The effect of process time and temperature on the accumulation of polar compounds in cottonseed oil during deep-fat frying. J. Sci. Food Agric., 83: 314-319.

Hsieh, C-C. and Lin, B-F. (2005).
The effects of vitamin E supplementation on autoimmune-prone New Zealand black X New Zealand white F1 mice fed an oxidized oil diet. British Journal of Nutrition, 93: 655-662.

Itaya, S. and Igarash, K. (1992).
Effect of taxifolin on the serum cholesterol level in rats. Biosci. Biotech . Biochem ., 56: 1492 – 1494 .

Izaki, Y., Yoshikawa, S. and Uchiyama, M. (1984).
Effect of ingestion of thermally oxidized frying oil on peroxidative criteria in rats. Lipids, 19: 324-331.

Jorge, N., Guaraldo, L. A. and Dobarganes M. C. (1997) :
Influence of fatty acid composition on the formation of polar glycerides and polar fatty acids in sunflower oils heated at frying temperature. Grasas Aceites , 48, 17-24.

Kerolles, Sh. Y. (2003).
Chemical and biological studies on some heated vegetables oils. Proc. The 1 st International Conf. " Food for Better Health", NCR , 18-20 October , Cairo , Egypt , 137-143.

Khan-Merchant, N.; Penumetcha, M.; Meilhac, O. and Parthasarthy (2002).
Oxidized fatty acids promote atherosclerosis only in the presence of dietary cholesterol in low – density lipoprotein receptor knockout mice. J. Nutr., 132: 3256-3262.

Kontush, A., Spranger, T., Reich, A., Djahansouzi, S., Karten, B., Braesen, J. H., Finckh, B., Kohlschutter, A. and Beisieegel, U. (1997).
Whole plasma oxidation assay as a measure of lipoprotein oxidizability . Biofactors 6, 99-109.

Liu, J.-F. and Huang, C.-J. (1995).
Tissue-tocopherol retention in male rats is compromised by feeding diets containing oxidized frying oil. J. Nutr., 125: 3071-3080.

Lopez-Varela, S., Sanchez-Muniz, F. J., Cuesta, C. (1995).
Decreased food efficiency ratio, growth retardation and changes in liver fatty acid composition in rats consuming thermally oxidized and polymerized sunflower oil used for frying. Food Chemistry and Toxicology 33: 181-189.

Manach , C., Regerat , F. and Texier, O. (1996).
Bioavailability, metabolism and physiological, impact 4- oxo flavonoids. Nutr. Res., 16, 517-544.

Marquez-Ruiz, G., Dobarganes, M.C. (1996).
Nutritional and physiological effects of used frying fats. In: Perkins, E. G., Erickson, M.C. (Eds.), Deep Drying . Nutritional and Practical Applications. American Oil Chemist s Society , Champaing , I L , pp. 427-429.

Meltzer, H. M., Folmer, M., Wang, S., Lie, Q., Maage, A. and Mundal, H. H. (1997).
Supplementary selenium influences the response to fatty acid induced oxidative stress in humans. Biological Trace Element Research 60: 51 – 67.

Naruszewicz, M.; Wozny, E.; Mirkiewicz, E.; Nowicka, G. and Szostak, W.B. (1987).
The effect of thermally oxidized Soya bean oil on metabolism of chylomicrons in cultured mouse macrophages. Atherosclerosis, 66: 45-53.

Nawar, W.W. (1979).In:
The chemistry of fats and oils. An overview short curse sponsored by food and nutrition press, Inc. West port C.T.

Orthoefer, F. T. and Cooper, D. S. (1996).
Evaluation of used frying oil: In Deep Frying Chemistry, Nutrition and Practical Application, Eds. E. G. Perkin, M. D. Erickson, AOCS Press, Champaign, IL (USA), pp. 285-310.

Ottolenghi, A. (1959).
Interaction of ascorbic acid and mitochondrial lipids. Arch. of Biochem. and Biophy., 79: 355-363.

Ozdil, S., Bolkent, S., Yanardag, R. and Arda-Pirincci, P. (2004).
Protective effects of Ascorbic acid, DL-α-tocopherol acetate, and sodium selenate on ethanol-induced liver damage of rats. Biological Trace Element Research, 97: 149-161.

Paul and Mittal, (1997).
Regulating the use of degraded oil/fat in deep-fat/oil food frying. Crit. Rev. Food Sci. Nutr., 37: 635-662.

Penumetcha, M., Khan, N. and Parthasarathy, S. (2000).
Dietary oxidized fatty acids: an atherogenic risk? J. Lipid Res., 41: 1473-1480.

Ramadan, M.F. Amer, M.M. A. and Sulieman, A. E. M. (2006).
Correlation between physicochemical analysis and radical – scavenging activity of vegetable oil blends as affected by frying of French fries . Eur. J. Lipid Sci. Technol., 108, 670 - 678.

Rec, G. (1972).
Determination of lactate dehydrogenase by enzymatic colourimetric methods. J. Clin. Chem. Biochem, 10: 182.

Recknagel, R. O. (1983).
Carbon tetrachloride hepatotoxicity , status qua and future prospects , Trands pharmacol . Sci . March, 129.

Rees, R. K. and Torlow, M. J. (1979).
The hepatotoxic actions of allyl format, Biochem., J. 104:757.

Richmond, N. (1973).
Clin. Chem., 19: 1350-1356. Romero, A., Bastida, S. and Sanchez-Muniz, F. J. (2006).Cyclic fatty acid monomer formation in domestic frying of frozen food in sunflower oil and high oleic acid sunflower oil without oil replenishment . Food and Chemical Toxicology, 44: 1674-1684.

Sanchez-Muniz, F. J., Bastida, S. (2003).
Frying oil discarding: Polar content vs. oligomer content determination. Forum of Nutrition, 56: 345-347.

Santos, A. A. A.; Silva, M. V. G.; Guerreiro, L. T. A.; Alves, M. V.; Bastos, V. L. F. C.; Bastos, J. C. and Kovary, K. (2002).
Influence of norbixin on plasma cholesterol associated lipoprotein, plasma arylesterase/ paraoxonase activity and hepatic lipid peroxidation of swiss mice on a high fat diet. Food Chem., 77: 393-399.

Schermer, S. (1967).
The blood morphology of laboratory animals. Lengmans, Green and Co. Ltd. PP. 350.

Schwenke, D. C. (1998).
Antioxidants and atherogenesis. J. Nutr. Biochem., 9: 424-445.

Slater, T. F. (1978).
Biochemical mechanisms of liver injure. Academic press, London.

Snedecor, G. W. and Cochran, W. G. (1980)"
.Statistical methods". Book, pp. 420.7th Ed. Iowa Stat. Univ. Press, Ames, Iowa, U.S.A..

Steinberg, D., Parthasarathy, S., Carew , T. E., Khoo , J. C. and Witztum, J. L. (1989).
Beyond cholesterol; modification of low – density lipoprotein that increases its atherogenicity. N. Engl. J. Med., 320, 915-924.

Strapans, I., Rapp, J. H., Pan, X. M., Hardman, D. A. and Feingold, K. R. (1996).
Oxidized lipids in the diet accelerate the development of fatty streaks . Arterioscler. Thromb. Vasc. Biol., 16: 533-538.

Strapans, I., Rapp, J. H., Pan, X. M., Kim, K. Y. and Feingold, K. R. (1994).
Oxidized lipids in the diet are a source of oxidized lipid in chylomicrons of human serum. Arterioscler. Thromb., 14: 1900-1905.

Sulzle, A., Hirch, F. and Eder, K. (2004).
Thermally oxidized dietary fat upregulates the expression of target genes of PPARα in rat liver. J. Nutr., 134: 1375-1383.

Suzuki, K.T. and Ogra,Y. (2002).
Metabolic pathway for selenium in the body: speciation by HPLC-ICP MS with enriched Se. Food Additives and Contaminants, 19: 974 -983.

Szasz, G., (1969).
Determination of γ- L-Y-Glutamyl transferase (γ -GT). Clin.Chem., 22,124 -136.

Tebib, K.; Rouanet, J. M. and Besancon, P. (1997).
Antioxidant effects of dietary polymeric grape seed tannins in tissues of rats fed a high cholesterol- vitamin E- deficient diet. Food Chemistry, 59 (1): 135-141.

Varley, H., Gewenlock, A. and Bell, M. (1980).
Practical Clinical Biochemistry. Vol. 1. 5th edn, pp. 741, 897. London: William Heinemen Medical. Books, Ltd.

Wallach, j. (1992).
Interpretation of Diagnostic Tests. A synopsis of aboratory Medicine, 5 th Ed. A little brown hand- book, Boston.

Walters, M. and Gerade, H. (1970).
Ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma. Microchem J., 15:231.

Wieland, H. and Seidel, D. (1983).
A fully enzymatic colorimetric determination of HDL-cholesterol in the serum. J. Nutr., 109: 760 – 766.

Witztum, J. L. and Steinberg, D. (1991).
Role of oxidized low density lipoprotein in athererogenesis . J. Clin. Invest., 88: 785 - 1792.

Wu, C. C.; S. L. Y.; Chen, H. W.; Kuo, W. W.; Tsai, S. J. and Lü, C. K. (2002).
Differential effect of garlic oil and its three major organosulfur components on the hepatic detoxification system in rats. J. Agric. Food Chem., 50: 378-383.

Wu, P. F. and Nawar, W. W. (1986).
A technique for monitoring the quality of used frying oils. J. Am. Oil Chem. Soc., 63 (10), 1363-1367.

Yokozawa, T.; Nakagawa, T. and Kitani, K. (2002).
Antioxidative activity of green tea polyphenol in cholesterol fed rats. J. Agric. Food Chem., 50: 3549- 3552.

Zamora, R., Hidalgo, J. and Tappel, A. A. (1991).
Comparative antioxidant effectiveness of dietary B-carotene, vitamine E, selenium and coenzyme Q10 in rat erythrocytes and plasma. J. Nutr., 121: 50-65.

تأثير مضادات الأكسدة على فئران التجارب المغذاه على الزيت المعامل حراريا (زيت التحمير)

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قسم الأغذية الخاصة والتغذية

الملخص العربى

في هذه الدراسة تم إخضاع زيت عباد الشمس لعمليات قلى متقطعة على درجة حرارة تتراوح من ١٨٠-١٩٠ °م لمدة ٢٤ ساعة وتم دراسة التغير فى الخواص الكميائية لهذا الزيت و مقارنته بزيت عباد الشمس الطازج (الكونترول) وأظهرت النتائج حدوث زيادة في الرقم الحمضى ورقم البيروكسيد والبوليمر وحدوث أنخفاض فى الرقم اليودى . كما كان ملاحظا أن التحمير أدى إلى حدوث تغير في نسبة الأحماض الغير مشبعة إلي المشبعة وذلك بمقارنة الزيوت المعاملة حراريا ً بالزيت الطازج (لم يتم معاملته حراريا ً). وباستخدام ٥٤ فار بوزن ١٠٠جرام تقريباً تم تقسيمهم إلى ٩ مجاميع وإحتوت كل مجموعة على ٦ فئران . المجموعتان (S2 و S3) تم تغذيتهما على عليقة تحتوى على زيوت معاملة حراريا ً بنسبة إضافة ١٥ % أما باقى المجاميع (S4 و S5 وS6 وS7 وS8 وS9) تم تغذيتهم على نفس العليقة المستخدمة فى تغذية المجموعة S2 ولكن تم تزويد العليقة بتركيزات مختلفة من مضادات الأكسدة مثل BHT (٠,٠٢ جرام /١٠٠جرام من الزيت) والصوديوم سلينيت بتركيز(١,٥ ملجم/ كجم من وزن العليقة) و البيتاكاروتين (١٠ملجم /٢٠٠ جم من وزن الفار/يوم) والأنثوسيانين بتركيز (١,٤٣ ملجم/٢٠٠جرام من وزن الفار/يوم) وزيت القرفة بتركيز (٠,٩ جرام/١٠٠جرام زيت) وزيت القرنفل بتركيز (٠,٩ جرام/١٠٠جرام زيت) وذلك على التوالى كل على حده. وأظهرت النتائج أن الفئران التى تم تغذيتها على زيوت معاملة حراريا ً (S2 و S3) بدون إضافة مضادات أكسدة إرتفاع فى تركيز الكولستيرول الكلى والكولستيرول منخفض الكثافة والجلسريدات الثلاثية وذلك مقارنة بالفئران التى تم تغذيتها على عليقة تحتوى على الزيوت المعاملة حراريا ومضاف له مضادات أكسدة مختلفة(S4 و S5 وS6 وS7 وS8 و S9). كذلك كان من الملاحظ إرتفاع فى تركيز هرمون الثيروكسين فى المجموعتان (S2 و S3) مقارنة بالمجاميع (S4 و S5 وS6 وS7 وS8 وS9) . و كان من الملاحظ حدوث تكسير لجدر الخلايا في المجموعتان (S2و S3) تم الأستدلال عليه من حدوث أرتفاع فى نشاط إنزيم اللاكتيك أسد ديهيدروجينيز (LDH) وكذلك زيادة فى معدلات الأكسدة TBARs (الثيوباربيوتريك أسد) وذلك مقارنة بالمجاميع الأخرى (S4 و S5 وS6 وS7 وS8 وS9) . و باستخدام بيوتلاتد هيدروكسى تولوين (BHT) كمضاد أكسدة صناعى بتركيز ٢٠٠ جزء في المليون أدى ذلك لحدوث إنخفاض فى معدلات الأكسدة TBARs و إرتفاع في نشاط إنزيم (LDH) وحدوث إرتفاع في نشاط وظائف الكبد والكلي. كذلك أوضحت الدراسة الهستولوجية لأنسجة (الكبد- الكلى- الغدة الدرقية – القلب) في الفئران التي تم تغذيتها علي الزيوت المعاملة حراريا ً (S2-S3) أن بها تغيرات واضحة غير مرغوبة للخلايا وذلك مقارنة بالفئران التي تم تغذيتها علي زيت طازج غير معامل (S1). كذلك أوضحت الدراسة الهستولوجية لأنسجة (الكبد- الكلى- الغدة الدرقية – القلب) للفئران التي تم إضافة مضادات الأكسدة الطبيعية المختلفة لها لم تحدث تغيرات لهذه الأنسجة كذلك كان من الملاحظ أن الفئران التي تم إضافة لها مادة BHT حدوث تدمير واضح لأنسجة الكبد- الكلى- الغدة الدرقية – القلب.