

## EFFECTS OF SOME EDIBLE OILS ON BLOOD COMPOSITION IN EXPERIMENTAL ANIMALS

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**ABSTRACT:** *The present work aims to study the potential effect of edible oils like olive, sunflower and flaxseed oils to give more protection against harmful effect of frying oil used in preparation of Tamiya (Falafel). Chemical and physical properties of oils were determined , also fatty acids composition of oils were identified . To study the harmful effect of oils used in preparation of falafel , twenty five of male albino rats were used over 45 days period. The animals were divided into (5) groups, wherein groups number (1) represent control which were fed on basal diet, while group number (2) was received 15% frying oil . Other three groups allowed to feed on high 15% frying oil with olive oil (5%) group number (3) , sunflower oil (5%) group number (4) and flaxseed oil (5%) group number (5). At the end of the experimental period, blood samples were collected to determine lipid profile include triglyceride , total cholesterol , HDL- cholesterol and LDL - cholesterol. The kidney functions include urea nitrogen and creatinine. From the obtained results , it concluded that group of rats fed on 15% frying oil were considered as a major risk factor for hyperlipidemia disease. The obtained results could be summarized that edible oils like olive oil , sunflower oil and flaxseed oil were considered the best for causing a reduction of TC, TG and LDL . Also, kidney function has been improved and there were a significant reduction urea than that of positive control group.*

**Key wards:** *Frying oils – Edible oils – Triglycerides - Cholesterol – kidney\_functions .*

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### INTRODUCTION

Tamiya (Falafel) is the most popular fried food product used by almost all Egyptians. It is a cheap, easily and quickly prepared, taste full and has a quite national value. Preparation of Tamiya in frying pastes contain broad bean , garlic , onion, Egyptian leek and spiced minced and frying in edible oil. Longtime and deep boiling of edible oil during the frying process gives to formation of free radicals and other harmful agents .

Frying is one of the oldest and simplest methods of food cooking, and it remains very popular. Frying involves putting food in contact with hot oil for various periods of time to withdraw the thermal energy and cook the food as mentioned by Rossell (2001). No matter

where or when frying originated, it remains a cooking method widely used domestically, commercially and industrially as mentioned by Chapman (2012). Frying is fast, generates unique flavors and textures which cannot be created by other means, and can be accomplished with simple equipment as mentioned by Rossell (2001). The frying of food can be performed in a variety of ways such as pan frying, deep fat frying, stir frying, and sauteing. Each type of frying can be modified to suit the needs of the individuals carrying out the frying process. Deep fat frying is an important food preparation process in the food industry because it is fast, convenient, and produces highly accepted foods amongst consumers. Unique properties of fats and oils provide a distinctive flavor, odor, and pleasing mouth-feel to

fry food products as mentioned by Orthoefer *et. al*, (2006a).

Due to oxygen absorption from the oil-air interface, leaching of food components, and the breakdown of oil constituents, a mixture of hundreds of chemical components is formed. The rapidly degrading frying oil can produce compounds that reduce oil quality, be absorbed by the fried food to create off-flavors, and cause a decrease in the nutritional value of the fried food as mentioned by Orthoefer *et. al*, (2006b). This can potentially become hazardous as highly oxidized oils have been linked to deleterious health effects in mice causing weight loss, liver and kidney damage, and intestinal tumours as mentioned by Paul *et al.*, (1997) and Stier, (2001). Three major types of reactions have been documented during frying: hydrolysis, oxidation and polymerization. Hydrolysis of ester bonds due to the moisture introduced by foods releases long-chain free fatty acids. Oxidation takes place due to the presence of air, and dimers and polymers are formed by radical recombination to form -C-C-, -C-OC-, and -C-O-O-C- bonds as mentioned by Sahin, and Sumnu (2009) , Gupta (2005) . The first health concern is the use of saturated frying oils such as animal fats. Saturated fats have been proven to increase low-density cholesterol (LDL) and reduce high-density cholesterol (HDL) as mentioned by Bockisch (1998). The changes in cholesterol level will promote the risk of cardiovascular disease such as hypertension, heart attack and stroke. Although initial studies focused on the overall Mediterranean diet, more recent studies compare a diet rich in olive oil to one low in olive oil. These studies provided a good evidence olive oil may be beneficial for reducing high blood pressure and preventing breast and

colon cancer. In vitro studies have been designed to identify how olive oil exerts its effects at the cellular level. The antioxidant capacity of olive oil contributes to many of its health benefits. The antioxidant action of olive oil in vitro has been highly documented and linked to such benefits as chemoprotection, anti-inflammatory action, and prevention of atherosclerotic plaque formation. as mentioned by Emily *et al.*, (2007).

Flaxseed oil or blends of flaxseed oil and sunflower oil promoted cholesterol reduction in hypercholesterolemic rats as compared to diets formulated with hard fats. These authors suggested that a diet with the appropriate balance of n-6 and n-3 fatty acids was preferred over diets high in n-6 fatty acids Ranhotra *et al.* (1992). Ground flaxseed is high in omega-3 fatty acids which have been shown to reduce hypertension, cholesterol and triglyceride level Oomah and Maza, (1998).

Sunflower oil is very high in polyunsaturated fatty acids and ranges between 64 and 68 percent. From a nutritional standpoint, this is desirable, because polyunsaturates are the source of essential fatty acids necessary for the production of prostaglandins. Prostaglandins play major roles in protecting the cardiovascular, reproductive, and immune and central nervous system Madhavi *et al.*, (2010).

## **MATERIALS AND METHODS**

### **Materials:**

The olive oil, sunflower oil and flaxseed oil samples were collected from local market. Tamiya boiled oil was collected from 3 different restaurants in Shebin El-Kom city, El-Minufia Governorates randomly.

**Methods :**

**Physico-chemical properties of the oils:**

The physico-chemical properties (saponification value, acid value, ester number, peroxide value, iodine value , specific gravity, viscosity and refractive index).of the oil were carried out using the method described by (A.O.A.C 1990).

**Identification of fatty acids:**

Saturated, unsaturated and total fatty acids were determined in the oil by using methyl esters boron trifluoride method A.O.A.C (2012) , the oil is saponified with sodium hydroxide in methanol .The fatty acids are methylised with boron tri fluoride in methanol, extracted with heptanes and determined on a gas chromatograph with FID detector (PE auto system XL) with auto sampler and Ezchrom integration system. Carrier gas (He), ca.25 Psi –air 450 ml/min –Hydrogen 45 ml – split 10 ml/min. oven temperature 200 ° C injector and detector 250 ° C.

**Biological Evaluation**

**Animals:**

Adult male albino rats Sprague Dawely strain weighing between (90 – 100 gm ) , were obtained from the animal house of Egyptian Organization for biological Products and Vaccines (VACSERA) Cairo, Egypt. The animals were kept in wire cages with wire bottom. The diet was introduced to the rats in special feed cup that kept food spilling to a minimum , water was provided to the rats by means of glass tube projecting through wire cage, an inverted bottle supported one side of the cage. The basal diet was formulated according to AIN - 93M, according to Reeves *et al.*, (1993). The used vitamin mixture component was that recommended by Campbell (1963) while the salt mixture used was formulated according to Hegsted (1941).

**Experimental Design:**

Twenty five rats were divided into five groups : group (A) control fed on basal diet, groups (B , C , D and E ) were allowed to feed 15% Tamiya boiled oil through the feeding period. One of each experiment continued feeding 15% Tamiya boiled oil without any supplementation saved as TBO group (B) and the other three groups of each experiment were allowed to feed 15% Tamiya boiled oil with olive oil (5%) as group (C) , sunflower oil (5%) as group (D) and flaxseed oil (5%) as group (E).

**Blood sampling and analysis**

Blood samples were collected after six weeks in tubes contain heparin as an anticoagulant from the eye plexuses under diethyl ether anesthesia and then centrifuged at 3000 rpm for 20 min. To obtain plasma, which was kept frozen until analysis. The total cholesterol was analyzed calorimetrically according to Allain *et al* (1974) method. HDL - cholesterol was determined according to Lopez *et al.* (1977) method . LDL-cholesterol was calculated according to formula of Kikuchi *et al.*, (1998). The triglycerides were analyzed according to Fossati and Prencipe (1982) method. Urea and creatinine were determined according to Young (2001 ).

**Statistical analysis**

The results of the animal experiments were expressed as the Mean ± SE and they were analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan’s test. In all cases  $p \leq 0.05$  was used as the criterion of statistical significance.

**RESULTS AND DISCUSSION**

Physical and chemical characteristics of olive oil , sunflower oil and flaxseed oil:

Table (1) showed the physico-chemical properties of edible oils. Acid values were 1.53 mg KOH/g for olive oil , 0.42 mg KOH/g for sunflower oil and 0.85 mg KOH/g for flaxseed oil. The acid value was lower than the Codex standard value for virgin vegetable oils (4.0 mg KOH/g Oil). The peroxide values were 3.27 Meq/kg for olive oil , 0.97 Meq/kg for sunflower oil and 2.64 Meq/kg for flaxseed oil. The value was lower than the codex standard value (up to 10 Meq/kg) for refined vegetable oil and lower than the maximum value (20 Meq/kg) allowed for unrefined olive oil (FAO/WHO, 1993). This implies that the oils have lower degree of rancidity. Also, iodine value were 74.14 , 137.1 and 201.93 for olive oil, sunflower oil and flaxseed oil, respectively . The lower iodine value signifies low degree of unsaturation and the lesser the liability of the oil to become rancid by oxidation. The saponification values were 198.18 mg KOH / g for olive oil , 196.89 mg KOH / g for sunflower oil and 202.53 mg KOH / g for flaxseed oil. The refractive index at 25 °C were 1.466 , 1.469 and 1.478 for olive oil , sunflower oil and flaxseed oil , respectively . Specific gravity and viscosity were 0.91 and 140 cp/s for olive oil , 0.925 and 130 cp/s for sunflower oil and 0.93 and 130 cp/s for flaxseed oil .

These results agreed with those previously reported by many other investigation Ibrahim (2000) and Firestone (1999), they found that Refractive index , acid number , iodine number and saponification number for flaxseed oil 1.4765 – 1.4779 , 1.57 - 1.78 mg/g , 189.7 - 202.3 g/100g and 195.3 - 203.5 mg KOH /g, respectively . Also Ibrahim *et al.*, (2000) reported , peroxide value ranged between 6.58 meq O<sub>2</sub>/kg and 10.14 meq O<sub>2</sub>/kg , saponification number from 187.05 mg KOH/ g to 194.85 mg KOH/ g . Iodine value ranged from (79.44 - 91.38 g of iodine/100 g oil), and

refractive index ranged from (1.4688 – 1.4702) , for olive oil.

#### Fatty acids composition:

As shown in Table ( 2 ) , the fatty acid profiles of olive oil , sunflower oil and flaxseed oil are presented . In fatty acids composition of olive oil . Only 3 saturated fatty acids from C16 to C20 and five unsaturated fatty acids from C18:1 to 20:1 including some highly unsaturated fatty acids such as C18:1 ω<sub>9</sub> (Oleic acid) , C18:2 ω<sub>6</sub> (Linoleic acid) and C18:3 ω<sub>3</sub> (linolenic acid) which recorded 74.25% , 6.82% and 0.65%, respectively .The fatty acids i.e. (PUFA) play an important role in human metabolic pathways, particularly as specific precursors for prostaglandin E1 Mendes *et. al.*, (2006). The unsaturated fatty acids are very important for the stability of oils because of the chemical reactions occurring at the double bonds. The rate of those oxidation reactions depend on the number of double bonds in the carbon chain. Therefore, olive oil with high proportion of oleic acid are more stable than the others. Also, oleic acid is less susceptible to oxidation than polyunsaturated fatty acid from the n-6 series (linoleic acid). The fatty acid profiles of sunflower oil , four saturated fatty acids from C16 to C21 and three unsaturated fatty acids from C18:1 to 18:2 including C18:1 ω<sub>9</sub> (oleic acid) , C18:1 ω<sub>7</sub> (Vaccinic acid ) and C18:2 ω<sub>6</sub> (linoleic acid) which recorded 27% , 1% and 61.86%, respectively. In fatty acids composition of flaxseed oil , two saturated fatty acids from C16 to C18 including palmitic and stearic acids which recorded 5.26% and 4.15% respectively , and four unsaturated fatty acids from C18:1 to 18:3 including C18:1 ω<sub>9</sub> (oleic acid) , C18:1 ω<sub>7</sub> (Vaccinic acid), C18:2 ω<sub>6</sub> (linoleic acid) and C18:3 ω<sub>3</sub> (linolenic acid) which recorded 17% , 0.87%, 15.6% and 57.65%, respectively.

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**Table (1): Physical and Chemical properties of oils**

Parameters	Olive oil	Sunflower oil	Flaxseed oil
Refractive index	1.466	1.469	1.478
Specific gravity	0.91	0.925	0.93
Viscosity cp/s	140	130	130
Acid value mg/g	1.53	0.42	0.85
Peroxide value Meq/kg	3.27	0.97	2.64
Saponification value mg KOH / g	198.18	196.89	202.53
Iodine value g/100g	74.14	137.1	201.93

**Table 2: Fatty acids composition of olive, sunflower and flaxseed oil**

Name of fatty acid	Relative distribution (%)		
	Olive oil	Sunflower oil	Flaxseed oil
Palmitic acid C16:0	11.16	5.93	5.26
Stearic acid C18:0	2.05	3.31	4.15
Oleic acid C18:1 ω9	74.25	27.0	17.0
Vaccinic acid C18:1 ω7	3.69	1.0	0.87
Linoleic acid C18:2 ω6	6.82	61.86	15.6
Arachidic acid C20:0	0.43	0.23	-----
Eicosaenoic acid C20:1ω11	0.31	-----	-----
Linolenic acid C18:3 ω3	0.65	-----	57.65
Behenic acid C21:0	-----	0.65	-----

Content of linoleic acid (C18:2) and linolenic acid (C18:3) as an essential fatty acid in the oils may be provide high nutritional remuneration and render beneficial healthy effect on blood lipid,

blood pressure and cholesterol contents Cheikh-Rouhou, *et al.* (2008) and it is preferred by industries when oil hydrogenation is required.

**In vivo study of the effect of frying oil and olive , sunflower and flaxseed oils on rats:**

Data in Table (3) indicates the level of plasma triglycerides, plasma total cholesterol , HDL- cholesterol and LDL-cholesterol of rats fed on basal diet , basal diet supplemented with 15% TBO , basal diet supplemented with 15% TBO + 5% olive oil , basal diet supplemented with 15% TBO + 5% sunflower oil and basal diet supplemented with 15% TBO + 5% flaxseed oil for 45 days. Data indicated that plasma triglycerides were  $175.4 \pm 3.3$  mg/dl after 45 days of feeding on 15% TBO while in normal group which feeding on standard diet triglycerides reached  $97.4 \pm 1.3$ mg/dl , meanwhile after 45 days of feeding on 15% TBO but contain 5% of olive oil , sunflower oil and flaxseed oil triglycerides reached  $119.4 \pm 3.5$ ,  $122.2 \pm 2.2$  and  $128.4 \pm 2.3$  mg/dl respectively. Plasma total cholesterol were  $82.4 \pm 6.1$  mg/dl after 45 days of feeding on 15% TBO while in normal group which feeding on standard diet total cholesterol reached  $68 \pm 3.8$  mg/dl , meanwhile after 45 days of feeding on 15% TBO but contain 5% of olive oil , sunflower oil and flaxseed oil total cholesterol reached  $73.8 \pm 5.3$  ,  $74.6 \pm 5.1$  and  $74.8 \pm 5.1$  mg/dl respectively. Plasma HDL-cholesterol were  $34 \pm 2.2$  mg/dl after 45 days of feeding on 15% TBO while in normal group which feeding on standard diet HDL-cholesterol reached  $42.2 \pm 1.9$  mg/dl, meanwhile after 45 days of feeding on 15% TBO but contain 5% of olive oil , sunflower oil and flaxseed oil HDL-cholesterol reached  $37.4 \pm 1.6$  ,  $35.4 \pm 2.6$  and  $36.6 \pm 2.6$  mg/dl respectively. Plasma LDL-cholesterol of rats were  $48.4 \pm 1.9$  mg/dl after 45 days of feeding on 15% TBO while in normal group which feeding on standard diet LDL-cholesterol reached  $25.8 \pm 1.3$  mg/dl , meanwhile after 45 days

of feeding on 15% TBO but contain 5% of olive oil , sunflower oil and flaxseed oil LDL-cholesterol reached  $36.4 \pm 1.8$  ,  $39.2 \pm 2.1$  and  $38.2 \pm 2.3$  mg/dl respectively.

These results are agreed with Craig, (1999), who mentioned that , a high ALA flaxseed diet was associated with reduced levels of TG and TC. Also, Vijaimohan *et al.*, (2006) mentioned that ALA supplementation significantly lowered the increased levels of plasma TC, TG, LDL-cholesterol, LDL/HDL-cholesterol, and TC/HDL-cholesterol . Also, Djousse *et al.*, (2003) stated that ALA intake at 0.81 and 0.69 g/d in men and women significantly reduced plasma TG levels.

Data in Table (4) indicates the level of plasma creatinine of rats .Plasma creatinine were  $0.9 \pm 0.1$  mg/dl after 45 days of feeding on 15% TBO while in normal group which feeding on standard diet creatinine reached  $0.86 \pm 0.11$  mg/dl , meanwhile after 45 days of feeding on 15% TBO but contain 5% of olive oil , sunflower oil and flaxseed oil creatinine reached  $0.8 \pm 0.07$  ,  $0.76 \pm 0.05$  and  $0.84 \pm 0.16$  mg/dl respectively. Plasma urea of rats were  $42.6 \pm 1.51$  mg/dl after 45 days of feeding on 15% TBO while in normal group which feeding on standard diet urea reached  $40.1$  mg/dl , meanwhile after 45 days of feeding on 15% TBO but contain 5% of olive oil , sunflower oil and flaxseed oil urea reached  $34.6 \pm 1.67$  ,  $41.8 \pm 0.83$  and  $39.8 \pm 1.48$  mg/dl respectively.

After 45 days , the supplementation of olive oil and flaxseed oil to basal diet containing TBO decreased significantly plasma urea to  $34.6 \pm 1.67$  and  $39.8 \pm 1.48$  mg/dl respectively, compared with TBO group  $42.6 \pm 1.51$  mg/dl.

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**Table (3): Effect of olive, sunflower and flaxseed oil on plasma triglycerides, total cholesterol, HDL and LDL-cholesterol in rats feeding Tamiya boiled oil for 45 days.**

Group	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL-C (mg/dl)	LDL--C (mg/dl)
Control	97.4±1.3 <sup>a</sup>	68±3.8 <sup>a</sup>	42.2±1.9 <sup>c</sup>	25.8 ± 1.3 <sup>a</sup>
TBO	175.4±3.3 <sup>d</sup>	82.4±6.1 <sup>b</sup>	34±2.2 <sup>a</sup>	48.4 ± 1.9 <sup>c</sup>
TBO + olive	119.4±3.5 <sup>b</sup>	73.8±5.3 <sup>a</sup>	37.4±1.6 <sup>b</sup>	36.4 ± 1.8 <sup>b</sup>
TBO+sunflower	122.2±2.2 <sup>b</sup>	74.6±5.1 <sup>a</sup>	35.4±2.6 <sup>ab</sup>	39.2 ± 2.1 <sup>b</sup>
TBO+flaxseed	128.4±2.3 <sup>c</sup>	74.8±5.1 <sup>a</sup>	36.6±2.6 <sup>ab</sup>	38.2 ± 2.3 <sup>b</sup>

Values represent means ± S.E obtained from 5 animals.

Means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at (P ≤ 0.05).

**Table (4): Effect of olive, sunflower and flaxseed oil on plasma creatinine and urea in rats feeding Tamiya boiled oil for 45 days**

Group	Creatinine mg/dl	urea mg/dl
Control	0.86±0.11 <sup>a</sup>	40.1±1.78 <sup>b</sup>
TBO	0.9±0.1 <sup>a</sup>	42.6±1.51 <sup>c</sup>
TBO + olive	0.8±0.07 <sup>a</sup>	34.6±1.67 <sup>a</sup>
TBO+sunflower	0.76±0.05 <sup>a</sup>	41.8±0.83 <sup>c</sup>
TBO+flaxseed	0.84±0.16 <sup>a</sup>	39.8±1.48 <sup>b</sup>

Values represent means ± S.E obtained from 5 animals.

Means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at (P ≤ 0.05).

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دراسات كيميائية حيوية على تأثيرات بعض الزيوت الغذائية على تركيب الدم  
في حيوانات التجارب

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الملخص العربي

تهدف هذه الدراسة الى مدى امكانيه استخدام بعض الزيوت النباتيه كزيت الزيتون وزيت عباد الشمس وزيت الكتان في تحسين الضرر المحتمل حدوثه من كثره تناول الفلاقل التي يتم تحميرها في زيوت تستخدم لفترات طويله في عمليه القلي . في هذه الدراسه تم دراسه الخواص الطبيعيه والكيميائيه وكذلك تفريد للاحماض الدهنيه للزيوت . استغرقت التجريه ٤٥ يوما وتم احضار ٣٠ فار من حيوانات التجارب وتقسيمهم ٥ فئران لكل مجموعه وتغذيتهم كالتى :-  
مجموعه ١ تتغذى على عليقه عاديه -مجموعه ٢ تغذت على عليقه بها ١٥ % زيت طعميه مغلى -مجموعه ٣ تغذت على عليقه بها ١٥ % زيت طعميه مغلى + ٥ % زيت زيتون - مجموعه ٤ تغذت على عليقه بها ١٥ % زيت طعميه مغلى + ٥ % زيت عباد الشمس - مجموعه ٥ تغذت على عليقه بها ١٥ % زيت طعميه مغلى + ٥ % زيت كتان .  
اوضحت النتائج المتحصل عليها ان التغذية على وجبه تحتوى على ١٥ % زيت تحمير ادت الى ارتفاع مستوى الليبيدات بالدم ( مستوى التراى جليسريد - الكوليسترول الكلى - مستوى LDL ) بينما ادت المعامله بالزيوت النباتيه (زيت الزيتون - زيت عباد الشمس- زيت الكتان ) الى خفض مستوى الليبيدات بالدم .

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