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Hypercholesterolemic Rats**

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المجلة العلمية المحكمة لدراسات وبحوث التربية النوعية

المجلد الخامس - العدد الأول - مسلسل العدد (٩) - يناير ٢٠١٩

رقم الإيداع بدار الكتب ٢٤٢٧٤ لسنة ٢٠١٦

ISSN-Print: 2356-8690 ISSN-Online: 2356-8690

موقع المجلة عبر بنك المعرفة المصري <https://jsezu.journals.ekb.eg>

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Abstract:

In the present study, pumpkin seed oil was used as a nutritional source of different bioactive compounds. Also, examined new insights on its roles that posed promising biological activities including organ functions manipulation, oxidant/antioxidant status improvement and obesity prevention will be in the scope of this study. Forty eight normal male albino rats ($140\pm 10g$) were randomly divided into eight groups (6 rats per each) as follow: group (1) fed on standard diet only and used as a negative control group, group (2) fed on experimental diet contains 10% Supplier oil , group (3) fed on experimental diet contains 20% supplier oil, group (4) fed on experimental diet contains 15% supplier oil + 5%

pumpkin oil, group (5) fed on experimental diet contains 10% supplier oil + 10 % pumpkin oil, group (6) fed on experimental diet contains 5% supplier oil + 15% pumpkin oil, group (7) fed on experimental diet contains 20% pumpkin oil and group (8) fed on experimental diet contains 10% pumpkin oil.

The results show that after 8 weeks of treatment with pumpkin oil significantly ($P \leq 0.05$) enhanced liver functions, and serum lipid profile compared with the control or vegetable oils studied. In conclusion, some vegetable oils could be used individually or in mixture, as functional foods for reducing the risks of liver disorders, and serum hypercholesterolemia. Pumpkin seed oil could be used beside soybean and sunflower oils, the major edible oils consumed in Egypt for lowering their saturated fat help to reduce heart disease in this country, even though their total saturated fatty acids composition is only in small quantities.

Keywords: Pumpkin seed oil, bioactive compounds, liver function, serum lipid profile.

Introduction:

Vegetable oils are the most important source of fat in the human diet. They are natural products of plant origin consisting of ester mixtures derived from glycerol with chains of fatty acid contain about 14 to 20 carbon atoms with different degrees of unsaturation (**Emmanuel and Mudiakeoghene, 2008**). Vegetable oil is very common, affordable and used by majority of people across the globe especially in the tropics. Its

use as an antidote to prevent some oxidative stress related diseases and a complication is advocated (**Ogugua and Ikejiaku, 2005**).

Pumpkin (*Cucurbitasp.*) has been known since the dawn of time. Today, pumpkins are widely cultivated as a food and for decorative purposes. Pumpkin seed contribute significantly to the nutrition of human population in many parts of the world. As mentioned by **Alfawaz, (2004)**, the main nutritionally relevant components of pumpkin seed are proteins (30–51 %) and oil (up to 40 %). They are also rich in carbohydrates (up to 10 %) and microelements as representatives of micronutrients (4 – 5 %).

Gohari et al., (2011) determined the chemical composition and physicochemical properties of pumpkin seeds and fatty acids of their oil and found that the seeds contained 41.59 % oil and 25.4 % protein. Moisture, crude fiber, total ash, and carbohydrate contents were 5.2 %, 5.34 %, 2.49 %, and 25.19 %, respectively. Differences in the chemical composition of pumpkin seed and its oil contents in various pumpkin species is predominantly attributed to its broad genetic diversity, growth and fertilization conditions and also to the harvest time (**Younis, 2000**).

The pumpkin seed oil is dark green in color. As reviewed by **Alfawaz, (2004)** and **Ahmed and Ravi (2017)**, the specific gravity, dynamic viscosity and refractive index of the pumpkin seed oil were 0.915, 93.659 and 1.4662, respectively. Acid value (mg KOH/g oil), peroxide value (meq O₂/kg oil), iodine value (g I₂/100 g oil), saponification number (mg KOH/g oil), and unsaponifiable matter content (%) of the extracted oil from pumpkin seeds were 0.78, 0.39, 10.85, 104.36, and 5.73, respectively.

Pumpkin oil contains a high amount of free fatty acids including four dominant fatty acids including oleic, linoleic, palmitic and stearic acids which are present with the relative distribution of 43.8%, 33.1%, 13.4% and 7.8% respectively i.e. representing 98 + 0.1% of the total fatty acids amount (**Badr *et al.*, 2011**).

Regarding the phytochemistry of pumpkin oil, total phenolics compounds (mg gallic acid/kg oil), total tocopherols (mg tocopherol/kg oil), total sterols (%), and waxes (%) were 66.27, 882.65, 1.86, and 1.58, respectively (**Gohari *et al.*, 2011**).

In addition, several triterpenes such as cucurbita-5, 24-dienol, α - and β -amyrin and sterols are present in the seeds of Cucurbita maxima (**Cattel *et al.*, 1979**). Also, **Murkovic *et al.*, (1996)** analyzed 100 different breeding lines and found g-tocopherol contents ranging from 41 to 620 mg/g dry seeds and a-tocopherol contents between 0 and 91 mg/g dry seeds. The contents of the b- and d-tocopherols were found to be very low, but sporadically (10 and 18 out of 100 breeding lines) reached 16 and 49 mg/g dry seeds, respectively. Vitamin A and various carotenoids have been detected in pumpkin seed oils as well as in the pressing residues of Styrian pumpkin seed oils. The predominant carotene found in Styrian pumpkin seed oil was lutein (71%) followed by b-carotene (12%) and cryptoxanthin by 5.3% (**Vogel, 1977**). The essential trace metals zinc in pumpkin seeds acts as an antioxidant which is attributed to its ability to neutralize free radical generation or directly engross the iron or copper binding sites of lipids, proteins, and DNA molecules (**Amara *et al.*, 2008**).

Pumpkin seeds have long been used for health benefits and the seed oil has been shown to contain active beneficial components that may protect from oxidative stress **Rezk and Darwish, 2012** reported that biochemical analysis in the serum revealed that pumpkin seed oil (PSO) can be used as a useful adjunct for maintaining the integrity of biochemical functions and restoring the original histological architecture of kidneys and testis after irradiation.

Multiple studies have demonstrated that an antioxidative property of pumpkin seed oil could improve the fertility and help in preventing arteriosclerosis, high blood pressure and heart diseases as well as in stimulating the metabolism of accumulated fats **Stevenson and Hurst (2007)** reported that pumpkin seed extracts have been used as an adjuvant for immunomodulation, reproductive health and therapeutic purposes for wide ranges of disease conditions.

Additionally, Omega 3 and 6 essential fatty acids in pumpkin seed oil are important for healthy brain and body functioning as well as preventing and improving bladder and prostate problems (**Ahmed et al., 2017**). Also, **Friederich et al., (2000)** demonstrated that an oral administration of 500–1000 mg/day of pumpkin oil for 12 weeks decreased the International Prostate Symptom Scores by 41.4% and more than 96% of the patients had no undesired side effects indicating that pumpkin oil significantly improved the urinary dysfunction in patients.

Phytosterols present in the pumpkin seed oil are also being studied for its role in lowering cholesterol levels. In addition, together with the high content of linoleic acid, sterols can help in the treatment of lipid–

associated disorders such as atherosclerosis. Pumpkin seed oil has been found to provide a significant source in tocopherols (vitamin E) in diets. Diets high in pumpkin seed oil have also been associated with lower level of gastric, breast, lung and colorectal cancer (**Huang *et al.*, 2004 and Murkovic, 2009**).

Also, pumpkin seed oil improved dyslipidaemia, with decreased VLDL-cholesterol and triglyceride levels and additional cholesterol-lowering effects were associated with its virgin over refined (**Morrison *et al.*, 2015**). **Bourre *et al.*, (2004)** have further brought the importance of oleic acid, a monounsaturated fatty acid also present in pumpkin in reducing the susceptibility of the testis and epididymis to lipid Peroxidation. Also, **Gundidza *et al.*, (2009)** found that pumpkin seeds improve sexual stimulation and intromission and ejaculatory latency.

On the other hand, **Oyeyemi *et al.*, (2008)** reported that pumpkin plant extract caused a significant reduction in sperm count with primary and secondary abnormalities by producing further zinc and protein. Therefore, pumpkin seed oil but not the plant extract has been used in preclinical studies to explore its role in both the prevention and treatment of infertility in male animal models. Study carried out by **Paulauskiene *et al.*, (2004)** have demonstrated that an antioxidative property of pumpkin seed extract could improve the fertility and help in preventing arteriosclerosis, high blood pressure and heart diseases as well as in stimulating the metabolism of accumulated fats.

the studies by **Bharti *et al.*, (2013)** have provided pharmacological evidence of tocopherol fraction of raw seeds of *Cucurbitapepo* L. as

possessing an anti-hyperglycemic property mediated via the interactions of its various components with multiple signaling targets that play crucial roles in diabetes mellitus (DM).

In the present study, pumpkin seed oil was used as nutritional supplements as a natural source of different bioactive compounds. Also, examined new insights on its roles that posed promising biological activities including organ functions manipulation, oxidant/antioxidant status improvement and obesity prevention will be in the scope this study.

Materials and Methods:

Materials:

Pumpkin (*Cucurbitapepo*) seed samples were purchased from Haraz Company for Trading Herbs and Medical Plants, Bab El-Khalek, Cairo, Egypt. Supplier oils were obtained from Commodity Supply Authority, Ministry of Supply and Internal Trade, Zagazig, Egypt. Chemicals: Casein, as a main source of protein from Morgan Company for Chemicals. Cairo, Egypt. Vitamins and salts mixtures, all organic solvents and other chemicals (analytical grade) were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.

Experimental animals: Normal male albino rats (140±10g) were obtained from Research Institute of Ophthalmology, Medical Analysis Department, and Giza, Egypt.

Methods:

Oil extraction by cold pressing:

Cold pressing was performed in a laboratory prototype apparatus in series in department of nutrition and food science, Faculty of Home Economics, Minoufiya University flow has already been achieved (**Martinez *et al.*, 2013**). The extracted oil was later separated from the sediment by centrifugation at 5000 rpm (Hettich Zentrifugen, model Universal 32R) for 10 min at 24°C and stored in a freezer at -20°C.

Preparation of oils extract:

Methanol extracts of the selected oils were prepared according to the method of **Amin *et al.*, (2004)** with some modifications. In brief, 20 g from oil +180 ml methanol [80% (v/v)] were homogenized and transferred to a beaker and stirred at 200 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 1 h at room temperature. The extract was then separated from the residue by filtration through Whatman No. 1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent of methanolic extract was removed under reduced pressure at 55°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). The yield of the extracts were weighted and used for biological experiments.

Chemical characteristics of vegetable oils:

Acid value (mg KOH/ 100 gm oil), peroxide value (Meq O₂/ kg oil), saponification value (mg KOH required to saponify 1 gm oil) and iodine value (gm iodine/100 gm fat) were determined using the methods of the **A.O.A.C. (2007)**.

Fatty acids composition:

Individual fatty acids were determined by gas chromatography (GC-4CM Shimodzu) according to the methods mentioned by **Farag *et al.*, (1986)**.

Total phenolic content:

The concentration of TPC in methanol extracts was measured using UV spectrophotometer (Jenway-UV-VIS spectrophotometer), based on a colorimetric oxidation/reduction reaction, as described by **Skerget *et al.* (2005)** using Folin-Ciocalteu reagent. 0.5 mL of diluted extract (10 mg in 10 mL solvent) was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 2 mL of Na₂CO₃ (75 g/L). The sample was incubated for 5 min at 50°C then cooled. For a control sample, 0.5 mL of distilled water was used. The absorbance was measured at 760 nm. TPC expressed as gallic acid equivalent (GAE) was calculated, and the results were expressed as an mg GAE g⁻¹ extract.

Antioxidant activities:

Scavenging activity method:

RSA of OSE was measured by bleaching of the purple colored solution of DPPH· according to **Hanato *et al.* (1988)**. One hundred µL of OSE (10 mg extract/10 mL solvent) was added to 3 mL of 0.1 mM DPPH· dissolved in ethanol. After the incubation period of 60 min at room temperature, the absorbance was determined against a control at 517 nm (**Gulcin *et al.*, 2004**). Percentage of antioxidant activity of DPPH was calculated as follows:

$$DPPH \cdot scavenging \ activity \ (%) = [(A_0 - A_1)/A_0] \times 100$$

Where, A₀ is the absorbance of the control reaction, and A₁ is the absorbance in the extract.

***β*-carotene bleaching method:**

Antioxidant activity of the selected oils extract and standards (*α*-tocopherol, BHA, and BHT; Sigma Chemical Co., St. Louis, Mo) was determined according to the *β*-carotene bleaching method following a modification of the procedure described by **Marco (1968)**.

Diets:

The basal diet prepared according to the following formula of **Reeves *et al.*, (1993)** as follow: 12.5% casein (82% protein), corn oil (10 %), vitamin mixture (1 %), mineral mixture (4 %), choline chloride (0.2 %), methionine (0.3 %), cellulose (5 %), and the remained is up to 100% corn starch. The vitamin mixture component was recommended by (**Campbell, 1963**), while the mineral mixture is formulate according to (**Hegsted *et al.*, 1941**).

Experimental design:

All biological experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council (**NRC, 1996**). Rats (n=48 rats), were housed individually in wire cages in a room maintained at 25 ± 2°C and kept under normal healthy conditions. All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period,

the rats were randomly divided into eight groups (6 rats per each) as follow:

Group (1) fed on standard diet only as a negative control (10 % corn oil),

Group (2) fed on experimental diet contains 10% supplier oil,

Group (3) fed on experimental diet contains 20% supplier oil,

Group (4) fed on experimental diet contains 15% supplier oil + 5% pumpkin oil.

Group (5) fed on experimental diet contains 10% supplier oil + 10 % pumpkin oil. Group (6) fed on experimental diet contains 5% supplier oil + 15% pumpkin oil.

Group (7) fed on experimental diet contains 20% pumpkin oil.

Group (8) fed on experimental diet contains 10 % pumpkin oil.

Biological evaluation:

During the period of the experiment, all rats were weighed once a week and the consumed diets were recorded everyday (daily feed intake). At the end of the experiment, biological evaluation of the experimental diets was carried out by determination of body weight gain (BWG) and feed efficiency ratio (FER) according to **Chapman *et al.*, (1959)**.

Blood sampling:

At the end of experiment period (28 days) blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were received into glass centrifuge tubes, containing oxalate solution (1.34 %) as

anticoagulant. After centrifugation at 3000 rpm for 10 min., plasma was with down and used for the analysis of blood lipid parameters. The erythrocyte residue was washed with three successive portions of sodium chloride solution (0.9 %) and then haemolysed with deionised water for 30 min. Haemolysate was then centrifuged at 30,000 rpm for 30 min. and the supernatant fractions was transferred to a clean test tube and analyzed of antioxidant enzymes (**Stroev and Makarova, 1989**).

Hematological parameters:

Liver functions:

Serum glutamic pyruvic transaminase (SGPT/ALT) and serum glutamic oxaloacetic transaminase (SGOT /AST) were measured in serum using the modified kinetic method of **Tietz (1976)** by using kits supplied by Biocon Company, Alkaline Phosphatase (ALP) activity was determined using modified kinetic method of **Vassault *et al.*, (1999)** by using kits supplied by Elitech Company and Bilirubin was determined in serum according to the method of **Cominacini *et al.*, (1983)** using specific kits purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt.

Blood lipids profile:

Triglycerides (TG), Total cholesterol (TC) and HDL-Cholesterol were determined in serum using specific kits purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt. Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol

(VLDL-c) were determined according to the equations of **Friedewald et al., (1972)** as follow:

$$\text{Very low density lipoprotein (VLDL cholesterol)} = TG/5$$

$$\text{LDL cholesterol} = \text{Total cholesterol} - \text{HDL cholesterol} - \text{VLDL cholesterol}$$

Statistical Analysis:

All measurements were done in triplicate and recorded as mean \pm SD. Statistical analysis was performed with the Student t-test and MINITAB 12 computer program (**Minitab Inc., State College, PA.USA, 2010**).

Results and Discussion:

Total phenolic of selected vegetable oils methanolic extract:

The total phenolic content of selected vegetable oils methanolic extracts were shown in Table (1). The highest total phenolic content was obtained in Pumpkin oil (5.66 ± 1.32 mg GAE.100 g⁻¹). Supply oil was characterized by the lowest total phenolic compound content (1.87 ± 0.56 GAE.100 g⁻¹). In similar study of **Gohari et al., (2011)**.total phenolics compounds in PSO was 66.27 mg gallic acid/kg. The levels of total phenolics in Pumpkin oil are higher than recorded in different similar oils. **Haiyan et al., (2007)** determined low level of total phenolics content in soybean oil (0.227 mg. 100g⁻¹). Also, **Mahmoud, (2017)** determined low level of total phenolics content in soybean, sunflower and palm oils which recorded 1.49 ± 0.36 , 1.26 ± 0.23 and 1.08 ± 0.17 mg GAE.100g⁻¹.

Folin-Ciocalteu reagent measures the ability of any mixture to reduce phosphomolybdic and phosphotungstic acids to a blue complex (**Swain**

and Hillis 1959). The presence of ascorbic acid or other very easily oxidized substances, not considered as phenolic compounds, may also result in the formation of blue color with Folin–Ciocalteu reagent, causing an overestimation of total phenolic content (Singleton *et al.*, 1999; and Shahidi and Naczk 2004). Folin reagents may be inappropriate for plant extracts with high levels of other easily oxidizable substances (Padda and Picha 2007).

Table (1): Total phenolic, antioxidant activity and chemical characteristics/fat constants of selected vegetable oils

Components	Concentrate	
Total phenolic compound content		
	Supply oil	Pumpkin oil
Total phenolic content(mg GAE.100 g ⁻¹)	1.87 ± 0.56	5.66 ± 1.32
Antioxidant activity		
	Supply oil	Pumpkin oil
DPPH scavenging (%)	24.92 ± 3.11	31.04 ± 4.52
EC50 (µg)	19.74 ± 2.87	12.53 ± 1.89
Antiradical powder (ARP)	3.21×10 ⁻²	7.11×10 ⁻²
Antioxidant activity (AA, %)	43.65 ±7.32	54.67 ±5.11
Chemical characteristics/fat constants		
	Supply oil	Pumpkin oil

Acid value (AV, mg KOH/g oil)	3.55 ± 0.45	1.45 ± 0.27
Peroxide value (PV, meq/kg oil)	1.02 ± 0.06	0.810 ± 0.17
Iodine value (IV, Hanus solution)	82.45 ± 3.98	134.45 ± 4.84
Saponification number (SN, mg KOH/g oil)	195.76 ± 4.96	186.32 ± 4.18

* Values (means ±SD) with different superscript letters are statistically significantly different ($P \leq 0.05$); GAE ,Gallic acid equivalent .ARP, antiradical power; EC_{50} , the amount of sample needed to decrease the initial DPPH concentration by 50%, AA measures by Folin–Ciocalteu reagent method

Antioxidant activity of selected vegetable oils methanolic extract:

The percentage of DPPH scavenged by antioxidants contained in oil extracts is shown in Table (1). From such data it could be noticed that all of the studied oil extracts scavenged DPPH. The methanolic extracts of the oils were characterized by statistically significant differences in their antioxidant activity measured by both DPPH and Folin–Ciocalteu methods.

The highest antioxidant activity was displayed by the extract obtained from Pumpkin seed oil was (31.04 ± 4.52) followed by supply oil ($24.92 \pm 3.11\%$). Supply oil methanolic extract possessed the lowest ARP calculated from the amount of sample needed to decrease the initial DPPH concentration by 50% (3.21×10^{-2}) while the best ARP was exhibited by

the extract obtained from PSO (7.11×10^{-2}). These results agree with the data reported by **Bardaa *et al*, (2016)**, who stated that PSO exhibit a highly antioxidant activity compared to a standard antioxidant (BHT).

The data of the present study with the others indicated that the antioxidant activities of different vegetable oils including PSO was affected by many factors including type of oil, extraction media/conditions and the type/ molecular structure of phenolics content (**Ramadan and Moersel 2006**)

The molecular structure of phenols is important for their antioxidant activity, as this activity is enhanced by the presence of a second hydroxyl or a methoxy group in the *ortho*- or *para*-position (**Laranijinha 2002**).

Rezk and Darwish, (2012) found that PSO have long been used for health benefits because its contain active beneficial components that may protect from oxidative stress. The oxidative stress represents one of the significant physiological factor including in many diseases such diabetes, cardiovascular, obesity and aging (**Ahmed and Ravie, 2017**).

Chemical characteristic/fat constants of selected vegetable oils:

The chemical characteristics (fat constants) of tested vegetable oils were shown in Table (1). The highest fat constants i.e. AV, PV and SN were obtained in Supply oil which recorded 3.55 ± 0.45 mg KOH/g oil, 1.02 ± 0.06 meq/kg oil and 195.76 ± 4.96 mg KOH/g oil, respectively.

Pumpkin oil was characterized by the lowest fat constants i.e. AV, PV and SN 1.45 ± 0.27 mg KOH/g oil, 0.810 ± 0.17 meq/kg oil and 186.32 ± 4.18 mg KOH/g oil, respectively. The fat constants (AV, PV and SN)

determined in different tested vegetable oils could be arranged as follows: Supply oil>Pumpkin oil. The opposite direction was observed for the IV.

In similar study, acid value (mg KOH/g oil), peroxide value (meq O₂/kg oil), iodine value (g I₂/100 g oil), saponification number (mg KOH/ g oil), and unsaponifiable matter content (%) of the extracted oil from pumpkin seeds were 0.78, 0.39, 10.85, 104.36, 190.69, and 5.73, respectively (**Gohari *et al.*, 2011**).

In different vegetable oils, **Mahmoud, (2017)** found that highest fat constants i.e. AV, PV and SN were obtained in Palm oil which recorded 3.89 ± 0.13 mg KOH/g oil, 1.02 ± 0.16 meq/kg oil and 197.30 ± 3.07 mg KOH/g oil, respectively. Sunflower oil was characterized by the lowest fat constants i.e. AV, PV and SN 3.12 ± 0.17 mg KOH/g oil, 0.74 ± 0.11 meq/kg oil and 183.76 ± 5.76 mg KOH/g oil, respectively. Also, such data are in accordance with that observed by **Serag El-Din, (2001)** and **El-Sharkawy (2011)**. Furthermore, the highest SN recorded of Supply oil due to its high content of short chain fatty acids compared with the Pumpkin oil.

Fatty acids composition of selected vegetable oils:

Gas-liquid chromatographic analysis for the methyl ester of tested vegetable oils samples were carried out to indicate their fatty acid composition related to the type of oil. The obtained data were illustrated in Table (2). Supply oil has higher saturated fatty acids (SFA, 36.30%) than Supply oil (22.42%). The opposite direction was observed with the unsaturated fatty acids (USFA's). Also, in Supply oil, palmitic acid (30.18%) and oleic acid (38.50%) are the major component acids along

with linoleic acid (24.45%) and only a trace amount of linolenic acid (0.45%). The low level of linoleic acid and virtual absence of linolenic acid make the oil relatively stable to oxidative deterioration (**Frank, 2002**).

On the other side, Pumpkin oil have total saturated fatty acids compositions are 22.42%. The major saturates in Pumpkin oil are palmitate and stearate. Also, Pumpkin oil contain 25.15% and 52.14% of the monounsaturated oleate and PUFA's, respectively.

In similar study, **Siano et al., (2016)** highlighted that saturated FA (SFA) and monounsaturated FA (MUFA) of *C. maxima* produced in southern Italy showed similar values (25.20% and 25.54%, respectively), while the polyunsaturated FA (PUFA) content was 48.14%.

Mahmoud, (2017) found that Palm oil has a balanced fatty acid composition in which the level of saturated fatty acids (SFA's) is almost equal to that of the unsaturated fatty acids (USFA's). Palmitic acid (43.18%) and oleic acid (38.97%) are the major component acids along with linoleic acid (11.32%) and only a trace amount of linolenic acid (0.25%).

On the other hand, soybean oil and sunflower oil are not saturated fat, their total saturated fatty acid composition are only 13.10 and 16.59%, respectively. The major saturates in soybean and sunflower oils are palmitate and stearate and Palmitate is responsible for about 70% and 50% of the total saturated fat in such oils, respectively.

Table 2. Fatty acids composition of selected vegetable oils

Fatty acids (%)		Supply oil	Pumpkin oil
Lauric	12.0	0.31	0.00
Myristic	14.0	1.14	0.23
Palmitic	16.0	30.18	14.76
Stearic	18.0	4.28	6.83
Arachidic	20.0	0.39	0.46
Behenic	22.0	0.00	0.14
Total SFA		36.30	22.42
Palmitoleic	16:1	0.30	0.19
Oleic	18:1	38.50	25.15
Linoleic	18:2	24.45	51.66
Linolenic	18:3	0.45	0.58
Gadoleic	20:1	0.00	0.00
Total MUFA		38.80	25.34
Total PUFA		24.90	52.24
Total USFA		63.70	77.58
USFA/SFA ratio		1.75	3.46

Biological Results:

Feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) of rats consumed the selected vegetable oils:

Feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) of rats consumed the selected vegetable oils were shown in Table (3). Data in could be noticed that the normal rats feeding with modified pellet were recorded 18.31 ± 1.45 (g/d), 41.03 ± 3.12 (g/28d) and 0.090 ± 0.021 for FI, BWG and FER, respectively.

The consumption of selected vegetable oils including 10% supplier oil, 20% supplier oil, 15% supplier oil + 5% pumpkin oil, 15% supplier oil + 5% pumpkin oil, 10% supplier oil + 10 % pumpkin oil, 5% supplier oil + 15% pumpkin oil, 20% pumpkin oil and 10% pumpkin oil showed significantly ($P \leq 0.05$) decreased in FI, BWG and FER compared with normal rats by the rate of -11.10, -13.32, -10.49, -9.95, -5.53, -3.37% and -1.77; -29.48, -32.14, -25.12, -23.58, -18.31, -7.39 and -4.69%; and -0.17, -0.18, -0.15, -0.14, -0.14, -0.07 and -0.05%, respectively.

According to the previous results the effect of selected vegetable oils potency on FI, BWG and FER compared to control (-) group have the following sequences: 20% pumpkin oil > 10% pumpkin oil > 5% supplier oil + 15% pumpkin oil > 10% supplier oil + 10 % pumpkin oil > 15% supplier oil + 5% pumpkin oil > 10% supplier oil > 20% supplier oil. The feeding of supplier oil (5 and 10%) was significantly affected in related to the FI and BWG. The opposite direction was recorded for the all pumpkin groups.

The different effects recorded amongst groups could be attributed to the differentiation in oils composition of minor components including, phenolics, vitamins, tocopherols, sterols, chlorophylls, trace metals etc. Such bioactive compounds were found in many functional foods including artichoke, milk thistle, turmeric, black-caraway seeds, oat seeds, parley seed , gum Arabic and *Lepidium sativum* Seeds and affected in FI, BWG and FER as blending to the basal diet of rats (Arshadi *et al.*, 2014, and Zamzami *et al.*, 2019).

Table (3): Feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) of rats consumed the selected vegetable oils

Groups	FI (g/d)		B W G (g/28d)		F E R	
	Mean \pm SD	% of change	Mean \pm SD	% of change	Mean \pm SD	% of change
Group (1): fed on standard diet contains 10% corn oil and used as a negative control group	18.31 \pm 1.45		41.03 \pm 3.12		0.090 \pm 0.01	
Group (2): fed on experimental diet	16.27 \pm 2.17	-11.10	28.93 \pm 4.21	-29.48	0.075 \pm 0.012	-0.17

contains 10% supplier oil							
Group (3): fed on experimental diet							
contains 20% supplier oil	15.87±1.98	-13.32	27.84±2,87	-32.14	0.073±0.24		-0.18
Group (4): fed on experimental diet							
contains 5% pumpkin +15% supplier oil.	16.39±1.43	-10.49	30.72±3.90	-25.12	0.077±0.01		-0.15
Group (5): fed on experimental diet							
contains 10% pumpkin oil + 10% supplier oil.	16.49±2.75	-9.95	31.35±4.11	-23.58	0.077±0.03		-0.14
Group (6): fed on experimental diet							
contains 15% pumpkin oil+ 5% supplier oil.	17.30±3.17	-5.53	33.51±2.45	-18.31	0.078±0.01		-0.14
Group (7): fed on experimental diet							
contains 20% pumpkin oil	17.69±2.67	-3.37	37.99±1.45	-7.39	0.084±0.04		-0.07

Group (8): fed on experimental diet contains 10% pumpkin oil.	17.98±1, 22	-1.77	39.10±3. 98	-4.69	0.086±0. 02	-0.05
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Each value represents mean \pm SD. Means in the same column with different letters are significantly different at $p \leq 0.05$.

Liver functions (liver enzymes activity, U/L) in rats consumed different vegetable oils :

Liver functions (liver enzymes activity, U/L) in rats consumed selected vegetable oils were shown in Table (4). From such data it could be noticed that the normal rats feeding with normal pellet were recorded 67.66 ± 5.32 , 22.26 ± 1.22 and 150.89 ± 7.09 U/L for AST, ALT and ALP, respectively. The consumption of selected vegetable oils including 10% supplier oil, 20% supplier oil, 15% supplier oil + 5% pumpkin oil, 15% supplier oil + 5% pumpkin oil, 10% supplier oil + 10% pumpkin oil, 5% supplier oil + 15% pumpkin oil, 20% pumpkin oil and 10% pumpkin oil showed significantly ($P \leq 0.05$) increased in AST, ALT and ALP compared to normal rats by the rate of 36.46, 45.89, 28.70, 20.08, 18.01, 8.11% and 5.60; 65.47, 75.58, 55.86, 47.56, 39.16, 7.83; and 3.33 %; and 17.70, 20.71, 16.36, 13.03, 8.06, 6.40 and 4.29 %, respectively.

According to the previous results the effect of selected vegetable oils potency on AST, ALT and ALP compared to control (-) group have the following sequences: 20% pumpkin oil > 10% pumpkin oil > 5% supplier oil + 15% pumpkin oil > 10% supplier oil + 10% pumpkin oil > 15% supplier oil + 5% pumpkin oil > 10% supplier oil > 20% supplier oil. The feeding of

supplier oil (5 and 10%) was significantly affected in related to the AST, ALT and ALP. The opposite direction was recorded for the all pumpkin groups. Unfortunately, there a dearth of information regarding the effect of PSO on liver function. Therefore, the comparison of the present data with the others is not easy task.

During hepatocellular damage, varieties of enzymes normally located in the cytosol are released into the blood flow. Their quantification in plasma is useful biomarkers of the extent and type of hepatocellular damage (**Pari and Murugan, 2004**). Serum ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, serum ALT is more specific to the liver, and is thus a better parameter for detecting liver injury (**Williamson et al., 1996**).

Data of the present results are in accordance with those of **Murayama et al. (2008)** and **Abozalam et al. (2016)**. It is clear that the increased enzymatic activities level of ALT was the most affected enzyme meanwhile ALP was the least one. **Giannini et al., (2005)** demonstrated that ALT is more sensitive test for hepatocellular damage than AST.

Vegetable oils under certain conditions impair the integrity, structure and function of the hepatocytes via its ROS, leading to defective secretion of the bile due to the damaged bile ducts consequently elevation of ALP level in the blood (**Tawfik et al., 2003**). The feeding of supplier oil (5 and 10%) was significantly affected in related to the AST, ALT and ALP. The opposite direction was recorded for the all pumpkin groups. The less non-significant effects recorded for pumpkin groups could be attributed to

their content of bioactive compounds/natural antioxidants including, phenolics, vitamins, tocopherols, sterols, chlorophylls, trace metals etc.

Such bioactive compounds were found in many functional foods including artichoke, milk thistle, turmeric, black-caraway seeds, oat seeds, parley seed, gum Arabic, *Lepidium sativum* Seeds and by-products plant parts and ameliorated the increase of hepatic enzymes AST, ALT and ALP activities induced by CCl₄ (Arshadi et al, 2014, Sayed Ahmed, 2016 and Zamzami *et al.*, 2019).

Such bioactive compounds have the ability as antioxidant to ameliorate hepatotoxicity/liver injuries by increase the level of GSH (biologic antioxidant) and reduction MDA (oxidative stress biomarker) in addition to improve the enzymatic activities of ALT, AST and ALP although it haven't reach to normal value (Abozalam et al, 2016, Sayed Ahmed, 2016 and Zamzami *et al.*, 2019).

On the other hand, supplier + pumpkin oils group gave a good suppression of liver functions enzymes activities when compared the supplier oil separately. It could be mean that a combination of these oils may be more efficient for reducing serum level of AST, ALT and ALP, the biomarkers of liver functions stress, because the interactive effects occurred by their content of different categories of bioactive compounds.

Table (4): Liver functions (liver enzymes activity, U/L) in rats consumed the selected vegetable oils

Groups	Aspartate aminotransferase (AST) μ/L		Alanine aminotransferase (ALT) μ/L		Alkaline phosphatase (ALP) μ/L	
	Mean \pm SD	% of change	Mean \pm SD	% of change	Mean \pm SD	% of change
Group (1): fed on standard diet contains 10% corn oil and used as a negative control group	67.66 \pm 5.32		22.26 \pm 1.22		150.89 \pm 7.09	
Group (2): fed on experimental diet contains 10% supplier oil	92.33 \pm 6.98	36.46	36.84 \pm 3.76	65.47	177.61 \pm 11.43	17.70
Group (3): fed on experimental diet contains 20% supplier oil	98.71 \pm 4.88	45.89	39.09 \pm 2.22	75.58	182.15 \pm 4.67	20.71
Group (4): fed on experimental diet contains 5% pumpkin +15% supplier oil.	87.08 \pm 5.32	28.70	34.70 \pm 1.89	55.86	175.58 \pm 6.87	16.36
Group (5): fed on experimental diet	81.24 \pm 2.09	20.08	32.85 \pm 3.10	47.56	170.55 \pm 5.23	13.03

contains 10% pumpkin oil + 10% supplier oil.						
Group (6): fed on experimental diet contains 15% pumpkin oil+ 5% supplier oil.	79.84± 7.87	18. 01	30.98± 2.67	39.16	163.06± 5.67	8.06
Group (7): fed on experimental diet contains 20% pumpkin oil	73.15± 4.05	8.1 1	24.00± 1.04	7.83	160.55± 1.98	6.40
Group (8): fed on experimental diet contains 10% pumpkin oil.	71.45± 1.45	5.6 0	23.00± 3.16	3.33	157.36± 6.71	4.29

Each value represents mean ± SD. Means in the same column with different letters are significantly different at $p \leq 0.05$.

Serum lipid profile in rats consumed selected vegetable oils:

Total cholesterol and triglycerides:

Serum lipid profile including total cholesterol and triglycerides in rats consumed selected vegetable oils was shown in Table (5). From such data it could be noticed that the normal rats feeding with normal pellet were recorded 99.95 ± 5.33 and 47.54 ± 2.45 for TC and TG levels, respectively.

The consumption of selected vegetable oils including 10% supplier oil, 20% supplier oil, 15% supplier oil + 5% pumpkin oil, 15% supplier oil +

5% pumpkin oil, 10% supplier oil + 10 % pumpkin oil, 5% supplier oil + 15% pumpkin oil, 20% pumpkin oil and 10% pumpkin oil showed significantly ($P \leq 0.05$) increased in TC and TG compared to normal rats by the rate of 27.97, 33.68, 20.61, 16.49, 14.56, 6.46 and 4.04%; and 38.25, 48.72, 30.73, 20.42, 16.66, 11.33 and 8.55%, respectively.

According to the previous results the effect of selected vegetable oils potency on TC and TG compared to control (-) group have the following sequences: 20% pumpkin oil >10% pumpkin oil>5% supplier oil + 15% pumpkin oil> 10% supplier oil + 10 % pumpkin oil>15% supplier oil + 5% pumpkin oil>10% supplier oil > 20% supplier oil. The feeding of supplier oil (5 and 10%) was significantly affected in related to the TC and TG. The opposite direction was recorded for the all pumpkin groups.

HDL-c, LDL-c and VLDL-c

Serum lipid profile including HDL-c, LDL-c and VLDL-c in rats consumed selected vegetable oils was shown in Table (6). From such data it could be noticed that the normal rats feeding with normal pellet were recorded 39.07 ± 2.11 , 51.37 ± 5.32 and 9.51 ± 0.67 (mg/dl) for HDL-c, LDL-c and VLDL-c, respectively.

The consumption of selected vegetable oils including 10% supplier oil, 20% supplier oil, 15% supplier oil + 5% pumpkin oil, 15% supplier oil + 5% pumpkin oil, 10% supplier oil + 10 % pumpkin oil, 5% supplier oil + 15% pumpkin oil, 20% pumpkin oil and 10% pumpkin oil showed significantly ($P \leq 0.05$) increased in LDL-c and VLDL-c compared to normal rats by the rate of 55.17, 68.67, 40.67, 32.31, 28.55, 10.68 and

4.25%; and 38.25, 48.72, 30.73, 20.42, 16.66, 11.33 and 8.55%, respectively.

According to the previous results the effect of selected vegetable oils potency on LDL-c and VLDL-c compared to control (-) group have the following sequences: 20% pumpkin oil >10% pumpkin oil>5% supplier oil + 15% pumpkin oil> 10% supplier oil + 10 % pumpkin oil>15% supplier oil + 5% pumpkin oil>10% supplier oil > 20% supplier oil.

The feeding of supplier oil (5 and 10%) was significantly affected in related to the LDL-c and VLDL-c but non-significant with all pumpkin groups. The opposite direction was recorded for the HDL-c.

Phytosterols present in the pumpkin seed oil are also being studied for their role in lowering cholesterol levels. In addition, together with the high content of linoleic acid, sterols can help in the treatment of lipid-associated disorders such as atherosclerosis. Pumpkin seed oil has been found to provide a significant source in tocopherols (vitamin E) in diets. Diets high in pumpkin seed oil have also been associated with lower level of gastric, breast, lung and colorectal cancer (**Huang *et al.*, 2004 and Murkovic, 2009**).

Morrison *et al.*, (2015) used pumpkin seed oil because of its high levels in unsaturated fatty acids and a rich source of phytochemicals. At the end, mice were fed with a western-type diet containing cocoa butter (15% w/w) and cholesterol (1% w/w) for 20 weeks to induce risk factors and disease endpoints. In separate groups, cocoa butter was replaced by refined or virgin pumpkin seed oil.

These studies demonstrated that both oils improved dyslipidaemia, with decreased VLDL-cholesterol and triglyceride levels compared to western-type diet, and additional cholesterol-lowering effects were associated with virgin over refined pumpkin seed oil. While refined oil did not affect the plasma inflammatory markers, virgin oil reduced the circulating levels of serum amyloid A and soluble vascular adhesion molecule-130.

Many authors indicated that saturated fat has more impact on raising blood cholesterol levels than anything else in the diet (**Dupont *et al.*, 1990, Hegsted *et al.*, 1993 and Earl *et al.*, 2005**). Such finding was confirmed by the data of the present study. Amongst the selected vegetable oils, supplier oil is a saturated fat i.e. rich in saturated fatty acids subsequently consumption induce significant ($p \leq 0.05$) raising in the TC, LDL-c and VLDL-c.

Consumption of too much saturated fat has been associated with the development of heart disease, some cancers, and other health problems (**Earl *et al.*, 2005**). Some researches has shown that high levels of dietary saturated fatty acids are related to increased cardiovascular diseases (CHD) and that dietary modification can lower plasma cholesterol (**Hegsted *et al.*, 1993**). The amount of total fat consumed, rather than the specific type of fat, has been positively associated with cancer risk (**Dupont *et al.*, 1990**).

On the other side, pumpkin is not saturated fat, their total saturated fatty acid composition are only 22.42%. Such as reported in our data, the major saturates in supplier and pumpkin is palmitate and stearate. Palmitate is responsible for about 30.18 of the total saturated fat in

supplier oil while represents 14.76% in pumpkin oil. Substitution of palmitate for carbohydrates or monounsaturates in the diet increased levels of serum LDL-c and total cholesterol (**DeMan, 1992**). Stearate has been found to be relatively neutral in its effects on blood lipids, and some researchers (**Earl et al., 2005**) showed that dietary stearate actually lowered serum LDL-c and total cholesterol levels; thus, many people recommend that this saturate not be included in the category of hypercholesterolemic acyl groups.

Also, supplier and pumpkin oils contain 38.50 and 25.15% of the monounsaturate oleate. Studies have shown that the oxidation rate of oleate is much slower than that of the polyunsaturates, linoleate and linolenate, which oxidize quickly and are the major contributors to the poor stability of these oils (**White, (2000)**).

A diet high in monounsaturated fatty acids may help to reduce elevated levels of total plasma cholesterol without reducing the HDL-c level (**Earl et al., 2005**). Furthermore, pumpkin oil is rich in polyunsaturated fatty acids which contain 77.58%. Unsaturated fats, classified as either monounsaturated or polyunsaturated, can help lower the cholesterol levels in blood when substituted for saturated fats (**Earl et al., 2005**).

Ingestion of approximately 1–2% of daily calories as linoleate is widely accepted as the amount needed to meet the essential fatty acid requirement of rodent species and humans (**Holman et al., 1991**). In the present study, pumpkin oil recorded 51.55% of linoleate. The physiological effects of linoleate have been well characterized. Various

deficiency symptoms include depressed growth, scaly dermatoses, increased skin permeability, fatty liver, kidney damage, and impaired reproduction.

The 8% of linolenate of soybean oil makes it not only an excellent source of essential fatty acids, but also a member of the n-3 fatty acid group. A number of health benefits have been associated with the consumption of foods or oils that contain n-3 fatty acids. These associations originally derived from epidemiological studies of Eskimos who consumed high levels of n-3 fatty acid from seals and cold water fish (Lands, 1986). These Eskimos were found to have a low incidence of heart disease and immune system diseases. Finally, large-scale epidemiological studies suggest that individuals at risk for CHD benefit from the consumption of plant- and marine-derived n-3 fatty acids (Lands, 1986).

Table (5): Serum lipid profile including total cholesterol and triglycerides (mg/dl) in rats consumed the selected vegetable oils

Groups	Total cholesterol (TC)		Triglycerides (TG)	
	Mean \pm SD	% of change	Mean \pm SD	% of change

– Group (1): fed on standard diet contains 10% corn oil and used as a negative control group	99.95±5.3 3		47.54±2 .45	
– Group (2): fed on experimental diet contains 10% supplier oil	127.91±3. 89	27.97	65.72±4 .76	38.25
– Group (3): fed on experimental diet contains 20% supplier oil	133.61±5. 76	33.68	70.70±6 .45	48.72
– Group (4): fed on experimental diet contains 5% pumpkin +15% supplier oil.	120.55±2. 90	20.61	62.15±6 ,98	30.73
– Group (5): fed on experimental diet contains 10% pumpkin oil + 10% supplier oil.	116.43±6. 98	16.49	57.25±3 .45	20.42
– Group (6): fed on experimental diet contains 15% pumpkin oil+ 5% supplier oil.	114.51±8. 11	14.56	55.46±5 .44	16.66
– Group (7): fed on experimental diet contains 20% pumpkin oil	106.41±3. 78	6.46	52.93±2 .08	11.33
– Group (8): fed on experimental diet contains 10% pumpkin oil.	103.99±10 .43	4.04	51.60±3 ,92	8.55

Each value represents mean ± SD. Means in the same column with different letters are significantly different at $p \leq 0.05$.

Table (6): Serum lipid profile including total HDL-c, LDL-c and VLDL-c (mg/dl) in rats consumed the selected vegetable oils

Groups	HDL-c		LDL-c		VLDL-c	
	Mean ±SD	% of cha nge	Mean ±SD	% of chan ge	Mean ±SD	% of cha nge
Group (1): fed on standard diet contains 10% corn oil and used as a negative control group	39.07 ±2.54		51.37± 4.30		9.51± 1.24	
Group (2): fed on experimental diet contains 10% supplier oil	35.62 ±2.26	8.83	79.71± 5.58	55.17	13.14 ±1.60	38.2 5
Group (3): fed on experimental diet contains 20% supplier oil	32.89 ±2.18	15.8 1	86.64± 6.22	68.67	14.14 ±1.68	48.7 2
Group (4): fed on experimental diet contains 5% pumpkin +15% supplier oil.	36.95 ±2.34	5.42	72.26± 5.80	40.67	12.43 ±1.14	30.7 3
Group (5): fed on experimental diet contains 10% pumpkin oil + 10% supplier oil.	37.69 ±2.59	3.53	67.96± 3,70	32.31	11.45 ±1.03	20.4 2

Group (6): fed on experimental diet contains 15% pumpkin oil+ 5% supplier oil.	38.22 ±2.88	2.17	66.03± 3.68	28.55	11.09 ±1.02	16.6 6
Group (7): fed on experimental diet contains 10% pumpkin oil	43.15 ±3.05	10.4 4	56.85± 2.62	10.68	10.58 ±1.02	11.3 3
Group (8): fed on experimental diet contains 20% pumpkin oil.	46.45 ±3.45	18.8 8	53.55± 2.26	4.25	10.32 ±1.05	8.55

Each value represents mean \pm SD. Means in the same column with different letters are significantly different at $p \leq 0.05$.

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تأثير زيت بذور اليقطين على الفئران المصابة بارتفاع نسبة كوليستيرول الدم

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الملخص

في هذه الدراسة تم استخدام زيت بذور اليقطين للوقاية من السمنة في فئران التجارب كما يلي. قسمت فئران التجارب (٤٨ من ذكور فئران الألبينو والتي تزن 140 ± 10 جرام) عشوائيا الى ٨ مجموعات (٦ فئران لكل مجموعة) كمايلي : المجموعة الاولى تم تغذيتها على الوجبة القياسية فقط كعينة مقارنة سالبة، المجموعة الثانية تم تغذيتها على الوجبة القياسية بالاضافة الى ١٠ % زيت تموين، المجموعة الثالثة تم تغذيتها على الوجبة القياسية بالاضافة الى ٢٠ % زيت تموين، المجموعة الرابعة تم تغذيتها على الوجبة القياسية بالاضافة الى ١٥ % زيت تموين و ٥ % زيت بذور اليقطين، المجموعة الخامسة تم تغذيتها على الوجبة القياسية بالاضافة الى ١٠ % زيت

تموين بالاضافة الى ١٠% زيت بذور اليقطين ,المجموعة السادسة تم تغذيتها على الوجبة القياسية بالاضافة الى ٥% زيت تموين بالاضافة الى ١٥% زيت بذور اليقطين , المجموعة السابعة تم تغذيتها على الوجبة القياسية بالاضافة الى ٢٠% زيت بذور اليقطين,المجموعة الثامنة تم تغذيتها على الوجبة القياسية بالاضافة الى ١٠% زيت بذور اليقطين .

أظهرت النتائج أنه بعد ٨ أسابيع من المعاملة بزيت بذور اليقطين تحسنت بشكل ملحوظ ($P \leq 0.05$) وظائف الكبد ، ومستوى الدهون في الدم مقارنة مع الكنترول والزيوت النباتية قيد الدراسة. الخلاصة ، يمكن أن تمثل بيانات هذه الدراسة وغيرها من الدراسات حجر الزاوية نحو استخدام بعض الزيوت النباتية ، بشكل فردي أو في خليط ، كأغذية وظيفية للحد من مخاطر اضطرابات الكبد ، وزيادة كوليستيرول في الدم. ويمكن استخدام زيت بذور اليقطين بجانب زيت فول الصويا وزيت عباد الشمس وهي زيوت الطعام الرئيسية المستهلكة في مصر ، حيث يمكن أن يساعد على خفض محتواها من الدهون المشبعة و تقليل الإصابة بأمراض القلب ، حيث أنه يحتوي على كميات قليلة من الزيوت المشبعة.