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Utilization of some vegetable oil blends rich in omega-3 fatty acids: Biological evaluation

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Abstract

The effect of feeding rats diet containing 9% of some prepared vegetable oil blends instead of corn oil for 6 months on body weight gain, weight of some selected internal organs, blood picture, lipid profile and liver histopathological changes were studied. Also, the oil blends were used for preparing microcapsules and omega fatty food products. The results indicated that the body weight gain of the rats was differed according to the type of oil and feeding period. Except liver, the weight of the other determined internal organs of the different rat groups were not significantly different. Plasma triglycerides level was ranged from 88.67 to 90.66 mg/dl. The value of LDL-cholesterol was relatively less in the plasma of the rats fed on diet containing canola oil with flaxseed oil (CF-2) blend rich in linolenic acid and less in oxidation stability, followed by olive oil with canola oil (OC-1), olive oil with flaxseed oil (OF) and corn oil, respectively. The less histological changes in the liver tissues were observed in the rats group fed on diet containing CF-2 oil blend followed by those containing OC-1 and OF then corn oil. Oxidation of prepared microcapsules oil caused a darkening in red color an increase in FFAs, PV, TBA, P-anisidine value and specific UV-absorbance. The results showed that the sensory properties of the fatty omega food products were acceptable by panelists.

Key words: Biological evaluation, Blends, Microcapsules, Omega-3, Utilization

Introduction

The increasing public awareness with personal health and fitness turned the attention of the nutritionists to investigate the role of various dietary fats in the maintenance of health and reduction of risk of chronic diseases such as heart diseases, cancer, hypertension, obesity and diabetes (Simopoulos, 1991).

Health and Welfare-Canada's Scientific Review Committee suggested that n-6 fatty acids (FAs) and n-3 FAs should provide at least 3% and 0.5% of energy, respectively with an n-6 to n-3 FAs ratio in the range of 4:1 to 10:1. The same ratio of n-6 to n-3 FAs was also recommended by World Health Organization (Richter, 2001). The factors affect this ratio are energy intake, total polyunsaturated fatty acids, absolute amount of n-6 and n-3 FAs in the diet (Maki et al., 2003). A lower ratio of n-6/ n-3 FAs is more desirable in reducing the risk of the chronic diseases of high prevalence in Western societies, as well as in the developing countries (Simopoulos, 1991; Gomez Candela et al., 2011). Consumption of recommended amounts of n-3 FAs can contribute to improvement of general health and welfare of entire population, especially young people (Sretenovic et al., 2009).

Both n-6 linoleic and n-3 linolenic acids interact with liver enzymes and convert to long chain fatty acids through a process of desaturation and elongation, respectively. The new produced fatty acids build hormone like eicosanoids that regulate immune and inflammatory responses. Such hormones form from 20 carbon atoms long and include prostaglandins, leukotrienes and thromboxanes (Fan and Chapkin, 1998). The parent fatty acids for eicosanoids are arachidonic acid (AA) from n-6 FAs family and eicosapentaenoic acid (EPA) from n-3 FAs family. The eicosanoids from n-6 FAs cause high risk of asthma allergic, arthritis, psoriasis, colitis and other inflammatory diseases. The functions of the eicosanoids of n-3 FAs have the opposite effects (Simopoulos, 1991). Therefore eating oils containing the proper ratio of n-6 to n-3 FAs will supply the body with essential fatty acids ideally for metabolism and will reduce the risk of host diseases including cardiovascular cancer, diabetes, obesity, arthritis, and asthma (Fan and Chapkin, 1998).

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In Egypt, the major edible vegetable oil sources are palm, soybean, cottonseed, sunflower and corn oils. Palm oil rich in saturated fatty acids and very low in n-3 FAs represents more than 70% of the total oil consumption. This was behind the increase of the cardiovascular diseases (CVD) and blood cholesterol between male and female in Egypt (Miladi, 1999). Increased n-3 FAs consumption can come from changes in diet. An alternative for increasing consumption would be to supplement with n-3 daily foodstuffs. Dietary supplements are a clear option for contributing to meet the established recommendation (Gomez Candela et al., 2011).

The aim of this study was to formulate vegetable oil blends rich in n-3 FAs and to evaluate their health claims in addition to estimate their palatability when use to prepare cold and warmed food products, as well as their storage stabilities when prepared in microcapsules form.

Materials and Methods

The following materials were obtained from Alexandria markets of Egypt, Netherland extra refined canola oil, cold press extra virgin olive oil (Wadifood Company, Egypt), fresh cold press crude flaxseed oil, refined corn oil (Arma Food Industries, Co., Egypt), Lurpak Danish Cow's butter (Dairy Aria Food Amba, Denmark), skimmed dry milk (Nestle Co., Egypt), minerals and vitamins mixtures (Altromin, D. 4937, Germany), baking powder and corn starch (Tag El-Melouk Co., Egypt), vinegar and mustard paste (Heinz Co., Egypt), 72% wheat flour extraction rate (Flour land Co., Egypt), sucrose powder (El-Doha, Co., Egypt), refined fine iodized common salt, fresh whole hen egg, with white shell, red cabbage, onion, yellow carrots, and lemon fruits.

Technological methods

Flaxseed oil pre-refining

The fresh crude pressed flaxseed oil was first degummed using 85% phosphoric acid, then neutralized with 15% sodium hydroxide, and bleached under vacuum at 90°C for 30 minutes using Tonsil ACCFF activated bleaching earth as described by Lillard (1982). The cooled bleached oil was packed in brown glass bottles and stored at 4°C until used.

Blending of oils

Olive oil (O) was individually mixed with each of canola (C) and flaxseed (F) oils and also canola (C) oil with flaxseed (F) oil at the following suggested ratios: 5.7 olive oil:1 canola oil (OC-1), 19 olive oil: 1 flaxseed oil (OF) and 9 canola oil : 1 flaxseed oil (CF-2). The blending was carried out at room temperature (22±2°C), then packed in brown glass bottles (100g) under stream of nitrogen gas. The oil bottles were kept at room temperature (22±2°C).

Encapsulation of oil blends

The CF-2 and OF oil blends, were embedded in gelatin capsules using centrifugal an extrusion encapsulation technique. This technique is based on utilizing nozzles consisting of concentric orifices located on the outer circumference of rotating cylinder. The encapsulating cylinder or head consists of a concentric food tube through which coating and core materials are pumped separately to the many nozzles mounted on the outer surface of the device. While the oil passes through the center tube, coating material flows through the outer tube (Schlameus, 1995).

Table 1. Recipes of the different omega food products prepared.

Ingredient in grams	Omega food products			
	Oil blend /butter	Mayonnaise	Fresh sauerkraut salad	Carrot cake
1-Butter	50	-	-	-
2-Oil blend*	50	125	9	75
3-Hen egg	-	10	-	20
4-Common salt	-	1.25	1.25	1.25
5-Mustard paste	-	4	2.5	-
6-Sucrose powdered	-	1	30	100
7-Lemon juice	-	20	-	-
8-Red cabbage	-	-	150	-
9-Red onion	-	-	10	-
10-Vinegar	-	-	50	-
11-Wheat flour	-	-	-	130
12-Baking powder	-	-	-	2.5
13-Grated carrots	-	-	-	150

*Both CF-2 and OF oil blends were used in preparing these products.

Preparation of omega food products

Table 1 shows the recipes of the different omega food products prepared in this study.

The preparation of the omega food products was done as described by Simopoulos and Robinson (1999). A- Oil blend/ Butter: Oil blend and butter were blended in food processor until combined and had a thick cream consistency then packed into a small polystyrene bowl, covered with aluminum foil and placed at 4°C to firm.

B- Mayonnaise: Twenty five grams of the oil blend plus the whole liquid egg, were placed into the bowl of the food processor and then salt, mustard paste and sucrose were blended until completely combined. To this mixture, the 50% of the remained oil blend was added in a very thin stream during the running of food processor then lemon juice was added with blending until combined. The final amount of oil blend was added and blended in a very thin stream as mentioned before. The obtained mayonnaise was packed in glass jar, covered with plastic cap and stored at 4°C until used.

C- Fresh sauerkraut salad: The cabbage and onion were sliced into thin slices. These slices were layered in the bottom of a bowl. The other ingredients (sucrose, vinegar, salt, mustard paste and oil blend) were mixed in a sauce pan, boiled and poured over the cabbage and onion layers while still hot. The resulted salad was kept at 4°C for 12 hours or more then occasionally stirred before presenting for taste panel testing.

D- Carrot cake: Oil blend, eggs and sugar were beaten in a mixer until obtaining a creamy texture. The dry ingredients were mixed in a separate bowl then added to the egg mixture and stirred until combined. The mixture was packed into a lightly greased baking pan and baked for about 35 minute at 350°C in a baking oven. After cooling at room temperature, it was packed in aluminum foil.

Analytical methods

Color of oil was assessed using Lovibond Tintometer, Model-E, 100000G, USA, using 0.5 inch cell (Mackinnery and Little, 1962). Free fatty acids (FFAs) as oleic acid, peroxide value (PV) as meq O_2/kg oil were determined as mentioned in AOCS (1985). Thiobarbituric acid (TBA) was colormetrically estimated at 538 nm as absorbance per kg oil (Pokorny et al., 1985) and P-anisidine value (P-An) was assayed at 350 nm as the absorbance resulting from the reaction of one gram oil with P-anisidine (Absorbance per g oil) (Egan et al., 1987). The values of specific absorbance of oil at K_{232} and K_{270} were determined as described by Kiritsakis (1991).

Biological methods

In this study, the experiments on animals were performed in accordance with the ethical guidelines and regulations set forth by experimental animal's laboratory, Home Economic Department, Faculty of Agriculture, Alexandria University. The experiments were done in accordance with the internationally accepted principles for laboratory animal use and care.

Sixty weanling male albino rats with a mean initial weight of 60±10 grams were divided into four groups of about equal weight. The rats in each group were hosted individually in metal cages. The rats were fed for a week as adaptation period on the control diet consisted of 37.5% skimmed milk, 30% corn starch, 13.5% powdered sucrose, 9% corn oil, 5% wood dust, 4% minerals and 1% vitamins (Abou-Arab and Hassan, 2006). After this period, the animals were switched to the experimental diets which contained instead of 9% corn oil, the same level from OC-1, OF and CF-2 oil blends. Each of control and experimental diets contained the following vitamins and minerals mixtures, 5.1g V.A, 0.0375g V. D2, 24g V. E., 1.2g V.K., 0.9g V. B_1, 1.5g V.B_2, 1.2g V. B_6, 6g V.C, 0.12g folic acid, 2.76g pantothenic acid, 6.0g nicotinic acid and 0.006g biotin per each one kilogram vitamin mixtures in lactose, the mineral mixture consisted of 33.4g Mn, 26.7g Zn, 20g Fe, 6.7g Cu, 0.83g I, 0.167g Co and 0.067g Sn per each one kilogram mineral mixtures in C_aCO_3. The feeding period on the experimental diet was continued for 12 weeks. Food and water were available to the animals during the experiment. Rat's body weight gain was determined through the feeding period (Abo-Arab and Hassan, 2006). At the end of the experiment, the animals were sacrificed after 14 hours of fasting. Weight of liver, kidney, spleen, heart, lung and testes were recorded and their ratios to body weight were calculated. Fresh liver kept in 10% formalin until histological assay performed.

Blood analysis: The blood sample was collected from each sacrificed animals and mixed with heparin as a blood anticoagulant in glass centrifugal tubes, left for 10 min then centrifuged at 8000 rpm for 10 min to separate serum from plasma. The obtained serum was kept at -20°C until analysis. Five samples of bloods of each group from five sacrificed animals were analyzed for: (**a**) Hemoglobin (Hb), according to Wintrobe (1965), using the following equation:

$$\text{Hb (g/dl)} = \frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times 36.77$$

(**b**) Red blood cell count (RBCs), using a light microscope at 40X magnification (Heplar, 1966); (**c**) White blood cell count (WBCs),using a light microscope at 25X magnification according to England and Bain (1976) method; (**d**) Hematocrit, according to Dacie and Lewis (1984), using the micro-method; (**e**) Calculation of Red cell values *Mean cell volume* (M.C.V.), *Mean corpuscular hemoglobin* (M.C.H.), *Mean cell hemoglobin concentration* (M.C.H.C.) according to Dacie and Lewis (1984); **(f**) Triglycerides (TG), according to the method of Fossati and Principle (1982), (**g**) total cholesterol (TCh) (Watson, 1960) as mg/dl using the following equation:

$$\text{Total cholesterol (mg/dl)} = \frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times 50$$

(**h**) High density lipoprotein (HDL) (Warnick et al., 1983), as mg/dl using the following equation:
HDL (mg/dl) = Optical density of sample × 220.

The methods of Warnick et al. (1983) were used to calculate LDL after measuring very low density lipoprotein (VLDL) using the following equations respectively,

VLDL = Triglycerides ÷ 5

LDL = Total cholesterol – (HDL + VLDL)

Histopathological examination: Three liver organ samples for each group were subscribed to histological measurement using haematoxylin and eosin (H & E) technique according to Drury and Willington (1983). Liver thin slices were stained by haematoxylin and eosin dyes. The slices viewed and photographed using Carlzeis light microscope.

Organoleptic methods

The omega food products were organoleptically evaluated using a set of 10 panelists from Food Science and Technology Department, Faculty of Agriculture, Alexandria Univ., Alexandria, Egypt. Each panelist was asked to evaluate the different sensory properties in each product according to the following methods:

a- Descriptive tests for oil blend / butter and carrot cake (Youssef, 1986; Abu-Foul, 1990).

b- Nine point hedonic scale ranging from 1 (Extremely dislike) to 9 (Extremely like) according to Gerczyca and Zabik (1979) for both mayonnaise and fresh sauerkraut salad.

Statistical methods

The data of the biological evaluation of oils were analyzed as a completely randomized design (Steel and Torrie, 1980) using the General Linear Model procedure of SAS (1986). Means were compared by Least Significant Difference (LSD) test (Steel and Torrie, 1980).

Results and Discussion

Biological effects of vegetable oil blends

In this part of the study the effect of feeding Albino male rats diet containing 9% of the prepared oil blends instead of corn oil for 3 months on their body weight gain, weight of some selected internal organs, blend picture, lipid profile and liver histopathological changes were studied.

Body weight gain

According to the data in Table 2, the body weight gain of the rats differed markedly due to type of oil in the rat feed and feeding period. Generally a significance gradual less in weight gain of the rats was noticed with extending feeding period. Such lower is mostly due to the less of rat appetite resulting from the oxidation of these types of vegetable oils.

The reduction in the body weight gain of the rats fed on OC-1 oil blend was noticed after the sixth weeks of feeding. The same observation was observed after 8 weeks in both group of rats which their diets contained OF oil blend and corn oil, the control group. These differences reflect the oxidation stability of these oils during feeding period and also the reaction of amino acids with the oxidized products of oils which caused a decrease of net utilization and digestibility of the protein, fat and fatty acids of feeds (Abou-Arab and Hassan, 2006).

Table 2. Changes in body weight gain in grams of rats feeding on oil blends*.

Type of oils	Feeding period (weeks)						
	1	2	4	6	8	10	12
CF-2	4.79^{c}±0.20	4.73^{c}±0.70	4.71^{d}±0.20	3.83^{d}±0.11	2.66^{c}±0.12	2.59^{d}±0.13	1.05^{d}±0.08
OC-1	9.94^{b}±0.09	9.89^{b}±0.17	8.26^{c}±0.36	5.29^{c}±0.19	4.03^{b}±0.09	3.01^{c}±0.08	1.63^{c}±0.11
OF	11.56^{a}±0.98	11.46^{a}±0.57	10.86^{a}±0.42	9.54^{a}±0.54	5.26^{a}±0.35	4.67^{a}±0.29	2.25^{b}±0.11
Corn oil	11.54^{a}±0.36	10.88^{a}±0.24	9.79^{b}±0.62	8.10^{b}±0.15	5.36^{a}±0.42	3.94^{b}±0.19	3.53^{a}±0.29

*Values are means ± standard deviation

The same letters in the same column were not significance.

Relative weight of some internal organs of rats

In the end of feeding experiment, the rats were slaughtered for measuring their organs weight. Results in Table 3 showed that, except liver, the weight of the others determined internal organs of the different rats groups feeding on different diets containing various types of oils were much closed. On the other hand, the rat liver weight was differed according to the type of oil in its diet. Rats group fed on diet containing corn oil (control diet) had the highest liver weight followed by those fed having CF-2, OC-1 and OF oil blends, respectively. According to Harman (1980) and Yetiv (1988), the negative approach of the intake of fat rich in polyunsaturated fatty acids (PUFA), is the risk of lipid peroxidation and free radical formation. The disorders of free radical formation include liver diseases, inflammation, malignancies, aging acceleration and atherosclerosis.

Blood picture

Table 3 showed that, the level of hemoglobin in the blood of the rats was very closed and ranged from 13.50 to 14.0 (g/dl). It lies in the normal hemoglobin range (13.5-18 g/dl) for male as reported by Tilkian et al. (1983). Generally, hemoglobin is an iron porphyrins attached to the globin protein. This conjugated protein able to combine reversibly with oxygen. The hem proteins function in oxygen binding, oxygen transport and electron transport. As seen from data in Table 3, the red blood cells (RBCs) or erythrocytes varied from 4.63 to 4.70×10^6 per cubic millimeter in the blood of the rats feeding diets having various types of oils. According to Tilkian et al. (1983) the normal count of RBCs of men ranges from 4.8 to 6.2×10^6/cmm. These cells are formed in bone marrow. The primary function of these cells is to transport oxygen to the tissues and to carry CO_2 to lungs. The white blood cells (WBCs) or leukocytes ranged from 8.17 to 8.30×10^3 per cubic millimeter in the blood of four groups of rats which feeding on diets having different oil types as stated in Table 3. This range is within the normal count of this type of cells ($4.5\text{-}11X10^3$/cmm) as stated by Tilkian et al. (1983).Also, the values reported in Table 3 for haematocrit, mean corpuscular hemoglobin (M.C.H), mean corpuscular hemoglobin concentration (M.C.H.C) and mean corpuscular volume (M.C.V) in the bloods of the four groups of rats feeding on diet having different types of oil were closed. But the mean corpuscular volume differed significantly in the bloods of the group of rats feeding on diet having OF oil blend and there were no significant differences within other groups. The normal value of M.C.H., M.C.H.C., M.C.V. and haematocrit in blood ranged from 26-32 pg, 32-36%, 78-94 FL and 34.0-40.0%, respectively (Heplar, 1966). The above results indicated that non-significant changes were observed among the blood pictures of the rats feeding on diets either having vegetable oil nearly free from n-3 fatty acids (corn oil) and/or containing various ratios of n-3 to n-6 fatty acids such as CF-2, OC-1 and OF oil blends.

Table 3. Changes in the relative weight of some organs, blood picture and lipid profile of rats feeding on oil blends*.

Property	Vegetable oil blends			Corn oil (Control)
	CF-2	OC-1	OF	
Weight of internal organs (grams)				
1- Liver	$3.61^b \pm 0.02$	$3.39^c \pm 0.1$	$3.27^c \pm 0.02$	$3.83^a \pm 0.03$
2- Kidney	$0.71^a \pm 0.03$	$0.76^a \pm 0.05$	$0.67^a \pm 0.08$	$0.70^a \pm 0.08$
3- Spleen	$0.40^a \pm 0.04$	$0.39^a \pm 0.06$	$0.39^a \pm 0.07$	$0.39^a \pm 0.02$
4- Lung	$0.90^a \pm 0.07$	$0.87^a \pm 0.02$	$0.95^a \pm 0.02$	$0.89^a \pm 0.07$
5- Heart	$0.34^a \pm 0.02$	$0.36^a \pm 0.03$	$0.35^a \pm 0.01$	$0.37^a \pm 0.02$
Blood picture				
Hb. (g/dl)	$13.9^a \pm 0.20$	$14.0^a \pm 0.70$	$13.9^a \pm 0.52$	$14.0^a \pm 0.60$
RBCs $10^6/mm^3$	$4.63^a \pm 2.06$	$4.63^a \pm 0.25$	$4.67^a \pm 0.25$	$4.70^a \pm 0.10$
WBCs $10^6/mm^3$	$8.30^a \pm 0.42$	$8.17^a \pm 0.21$	$8.20^a \pm 2.40$	$8.30^a \pm 0.10$
Haematocrit (%)	$41.56^a \pm 2.60$	$41.53^a \pm 2.54$	$41.40^a \pm 2.70$	$41.9^a \pm 2.04$
M.C.V (FL)	$89.76^a \pm 0.01$	$89.69^a \pm 0.15$	$88.65^b \pm 0.52$	$89.44^a \pm 0.35$
M.C.H (Pg)	$29.16^a \pm 0.20$	$30.24^a \pm 0.61$	$29.76^a \pm 0.20$	$24.78^a \pm 0.10$
M.C.H.C. (%)	$32.48^b \pm 0.20$	$33.71^a \pm 0.25$	$33.57^a \pm 0.30$	$33.11^a \pm 0.30$
Lipid profile (mg/dl)				
Total triglyceride	$88.67^a \pm 0.58$	$90.0^a \pm 3.00$	$89.66^a \pm 3.06$	$90.66^a \pm 1.16$
Total cholesterol	$178^a \pm 3.00$	$174^a \pm 2.60$	$178^a \pm 2.65$	$144^b \pm 4.00$
LDL-Ch.	$144.59^a \pm 1.53$	$147.0^a \pm 2.08$	$148.41^a \pm 1.93$	$110.87^b \pm 2.08$
HDL-Ch.	$15.67^a \pm 0.58$	$14.0^a \pm 1.00$	$14.66^a \pm 0.56$	$15.0^a \pm 2.65$

*Values are means ± standard deviation; The same letters in the same line were not significance

Table 4. Change of oil blends quality in the microcapsules during storage* (22±2°C).

Constitutes	Oils blend in microcapsules					
	CF-2			OF		
	Zero time	3 months	6 months	Zero time	3 months	6 months
Red color value	2.28	2.33	2.15	1.12	1.34	1.84
Free fatty acids (FFAs) as % oleic acid	0.34±0.04	0.51±0.01	0.73±0.05	0.24±0.04	0.49±0.06	0.74±0.05
Peroxide value (PV) as meq O_2/kg	2.17±0.08	5.88±0.02	9.58±0.04	2.07±0.06	4.86±0.15	7.14±0.07
Thiobarbituric acid (TBA) as absorbance /kg	0.031±0.00	0.115±0.00	0.498±0.01	0.016±0.01	0.040±0.00	0.374±0.03
P-anisidine value (P-Av) as absorbance / g	1.3±0.22	3.73±0.24	5.95±0.10	0.826±0.00	1.45±0.02	2.41±0.12
Absorbance values at K_{232}	0.52±0.03	1.21±0.09	1.89±0.14	0.51±0.02	0.95±0.04	1.59±0.04
Absorbance values at K_{270}	0.028±0.01	0.060±0.00	0.092±0.01	0.026±0.00	0.048±0.01	0.060±0.00

*Values are means ± standard deviation.

Lipid profile

The lipid profile comprises triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol. This group of tests is used in combination with factors such as age, blood pressures, obesity, smoking, diabetes and family history to assess coronary heart disease (CHD) risk and to select and monitor treatment (Dietscky et al., 1993). Data in Table 3 showed that the plasma triglycerides level ranged from 88.67 to 90.66 (mg/dl). It was slightly higher in plasma of the rats feeding on diet containing the free n-3 fatty acids oil, corn oil, than those having vegetable oil blends containing n-3 and n-6 fatty acids. The variation in this test did not vary significantly among the rats feeding on different diets. Generally, the values of the triglycerides in this table are within the normal range (35-165 mg/dl) of this component in plasma (Ismael et al., 2007). It is also cleared from the results in this Table that using rat diet containing oil blends instead of corn oil, caused a significant rise of the total cholesterol in the rat plasma. On the other side, this increase did not go as far as the upper limit of this component in human (200-250 mg/dl) (Taneja and Rakha, 2005). The value of LDL-cholesterol was more noticeable in the plasma of the rats feeding on diet containing OF vegetable oil blend, followed by OC-1, CF-2 and corn oil respectively. On the contrary, the HDL-cholesterol levels in plasma of the rats feeding on the diet containing different types of oils were much closed. The above results showed that the levels of total, LDL and HDL-cholesterol in the plasma profile of the rats did not only depend on n-3 to n-6 fatty acid ratio but also on the oxidation stability of the oil in their diets. According to Taneja and Rakha (2005) the presence of vitamin E with omega-3 fatty acids prevent the changes in blood lipid profile and impose no serious risk to the consumer. Ramirez-Tartosa et al. (1999) found that the consumption of olive oil plus fish oil caused a significant reduction in the plasma triglycerides compared with olive oil. Vegetable oil rich in the n-6 FAs have been promoted as lowering blood cholesterol concentration of the people in USA (Hass, 1999). Harris (1997) showed that the plant n-3 FA caused rise of LDL-cholesterol by 5-10% and HDL-cholesterol by 1-3%. Fickova et al. (1998) noticed that rats fed on the diet containing n-3 FA contained significantly lower level serum triglycerides compared with those fed on diets having n-6 FAs. The study of Mc-Donald et al. (1989) revealed that the diet containing canola oil or sunflower oil produced similar decrease in plasma total and low-density lipoprotein cholesterol and nearly no changes in both HDL-cholesterol and triglycerides. Pang et al. (1998) concluded from their research on replacement of linoleic acid with α-linolenic acid that both acids offer similar cardioprotective benefits with respect to lipid metabolism.

Histopathological of rat liver

Histology helps in the interpretation of the microscopic changes that occur under different pathological conditions. In this study, the liver tissues of the four groups of rats fed on diet containing 9% fat from different oil sources were microscopically examined. The results of such examination were illustrated in Figures 1 and 2. It is known that liver is one of the most important and large organs in the body. Generally it consists of hepatocytes, Von kupffer cells and blood sinusoids which transfer blood from the portal vein and hepatic artery to the central vein. The hepatocytes arrange in an anastomosing plates or laminates between the portal triad and the central vein or venule. It has polyhedral surface and contains mono or binucleus. The portal triad consists of three

branches, portal vein branch, hepatic artery branch and the bile duct branch. The three branches are surrounded with an indented collagen layer in the portal canal which has also the lymphatic vessels. Figure 1 show the liver tissues of the rats groups fed three months on diet containing 9% corn oil free from n-3 fatty acids. The following alterations were noticed in their liver tissues, (1) Enlargement in some hepatocytes, (2) Formation of some vacuolation in the intracytoplasm, (3) Dilation in portal tract and central vein, (4) Infiltration in portal tract within blood sinusoid, (5) Marked inflammation around portal tract and (6) Congestion in blood portal tract and central vein.

The influence of the diet containing CF-2 oil blend on the liver tissues of the rats was illustrated in Figure 1B. The examination of these figure showed only slight dilation in the blood sinusoid and mild inflammation with slight infiltration in portal tract. Nearly the liver tissues were normal.

The alteration in the liver tissues of the rats group fed on the diet having OC-1 oil blend included the following as shown from Figure 2A, (1) Mild dilation in the blood sinusoid, (2) Mild dilation in the tributary veins, (3) Market inflammation around portal tract and (4) Slight congestion in central vein.

In case of feed having OF oil blend, the main changes in the liver tissues of the rats were: (1) Enlargement in the hepatocytes, (2) Formation of few numbers of vacuolation in the cytoplasmic of the liver cells, (3) Mild dilation in the blood sinusoid as illustrated in Figure 2B.

The above results showed the important of the presence of n-3 fatty acids in the diet to reduce from the changes in the liver tissues. The less histological changes in the liver tissues was observed in the rats group fed on diet containing CF-2 oil blend followed by those containing OC-1 and OF then corn oil. These types of oils are easily metabolized in liver. The liver does not normally store fat, but it plays an essential role in the continual and rapid turnover of phospholipids, the saturation and desaturation of fatty acids, the oxidation and also the synthesis of fatty acids (Alwayn et al., 2005). It can be concluded from the data of the biological effect of oils on rats that both source and composition of oil in the diet had an effect on the weight gain, liver weight, triglycerides, total cholesterol, LDL-cholesterol of the plasma and histopathology of the liver tissues of the rats.

Utilization of oil blends

In this part of the study, the CF-2 and OF oil blends were used in preparing, microcapsules to use as a food supplement, omega fatty food products such as, oil blend/ cow butter and mayonnaise, fresh sauerkraut salad and carrot cake.

The following points were undertaken during preparing such products: 1-the products should be relatively low in saturated fat and omega-6-fatty acids; 2-the overall ratio of n-6 to n-3 fatty acids in these products is kept between 4-10: 1 as recommended by World Health Organization (Kris-Etherton et al., 2000), and n-3 fatty acids are found in all products; 3-the other prepared products than both fatty foods and the microcapsules are relatively low in calories; 4-such products should be simply, easily and rapid prepared.

Microcapsules

The encapsulation technique was used to prepare gelatin microcapsules containing CF-2 or OF oil blend. Each capsule contained 0.2 mg of oil blend. The prepared capsules were packed in a brown glass jar and kept at room temperature (22±2°C) for 6 months. The changes in FFAs, PV, TBA, P-AV and specific UV-absorption at 232 and 270 nm were determined to determine the oil stability in these capsules during storage period. The results were present in Table 4 and revealed that both composition of oil blend and storage period affected the quality of oil in the microcapsules. The rate of the changes in the determined parameters was more noticeable in CF-2 oil microcapsules than the OF ones. Because both blends containing flaxseed oil, the differences changes in the rate of the oil stability inside the capsules could be attributed to the other oil type in such blends. Canola oil had more unsaturated fatty acids especially linolenic acid than olive oil rich in oleic acid and nearly free from linolenic one (Sharara, 1998). Therefore, the rate of the oxidation was more expected in CF-2 blend than OF one. Also, extending the storage period to 6 months was associated with an increase in the rate of oil oxidation in microcapsules. The results of oil oxidation caused a darken in red oil color, an increase in FFAs, PV, TBA, P-AV, specific UV-absorbance at 232 and 270 nm particularly in microcapsules containing CF-2 oil blend. Generally, the rise in FFAs and PV values were less than the limits at which the oil should be rejected, (2% FFAs as oleic acid, and 10 meq O_2/kg oil PV value), respectively.

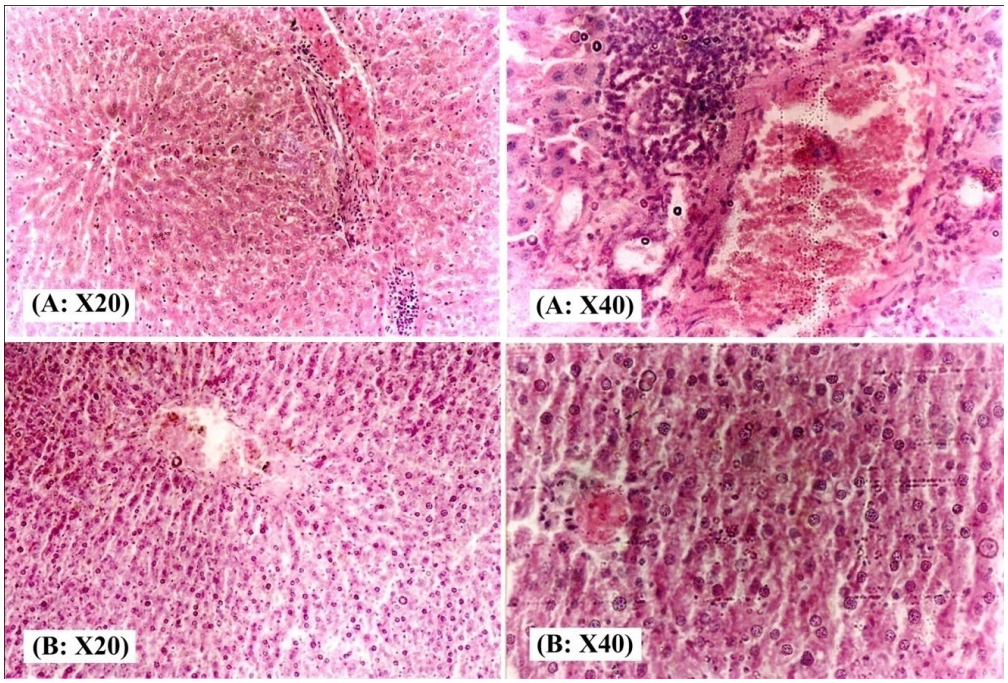

Figure 1. Light microscopic photograph of rat liver tissues.
A. Feeding on control; B. Feeding on CF-2 oil blend diet

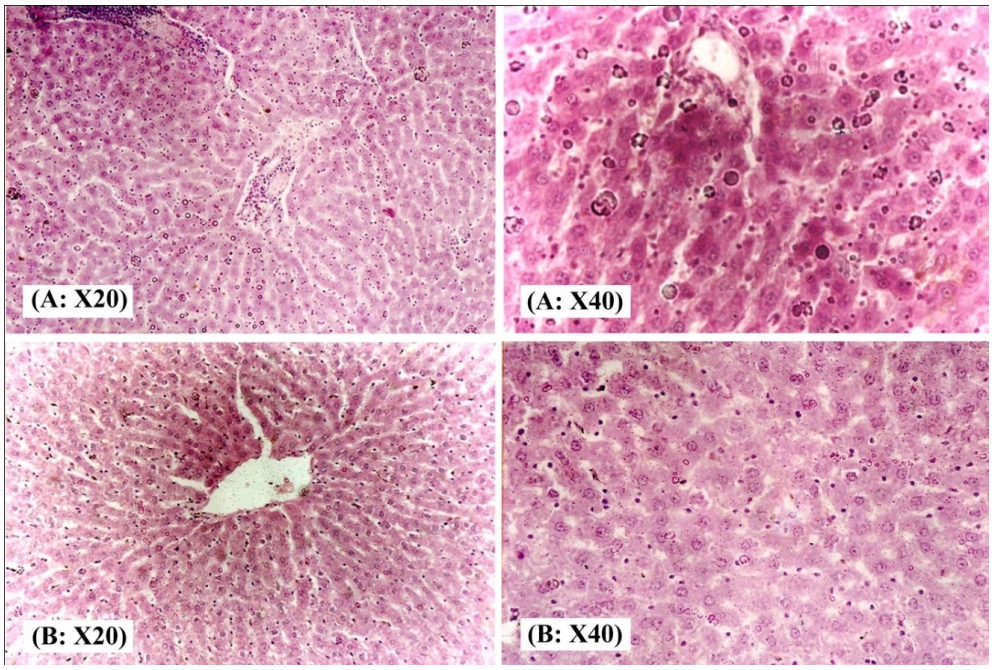

Figure 2. Light microscopic photograph of rat liver tissues.
A. Feeding on OC-1 oil blend diet; B. Feeding on OF oil blend diet

According to these results, the shelf life of such food supplement product can be recommended to be less than 6 months. The importance of such capsules is due to its high content of n-3 fatty acids which are an essential for brain function, hormone production and the other body functions. Also, the capsules provide a convenient way to cover the personal need from the right fat and its optimum level of the required EFAs. The daily need from oil blend capsules will depend on the total energy

intake, total polyunsaturated fatty acids intake and the absolute amount of n-6 to n- 3 fatty acids in the diet. According to Nielsen (1992), Denmark was the first country in the world which using microcapsules containing fish oil. Such capsules are used in preparing omega bread.

Omega fatty food products

(1) Oil blend / cow butter product: This product has about 50% of the saturated fat in cow butter and unlike margarine; it has a negligible amount of hydrogenated fat. Also it has a perfect consistency for spreading on toast or bread when uses after cooling and removing from refrigerator.

The color of this product was yellow and its flavor was an incipiently rancid. The acceptability of the color and flavor of such product were described by panelists as an excellent and good, respectively. No variations were noticed by panelists in the previous two characteristics between the products containing either CF-2 and/or OF oil blends. In contrast, the consistency of the product containing CF-2 oil blend was moderately spreading than that having the OF oil blend, slightly spreading. This can be attributed to the differences in the level and type of unsaturated fatty acids in the two oil blends. Therefore, the panelists described the consistency of the product containing CF-2 oil blend as good and the other product as fair. Generally leaving this product at room temperature for an hour or more caused a conversion of its consistency from plastic to nearly liquid. This problem can be solved by adding oil stabilizer to prevent the oil and butter from separating. Also, the flavor of such product can improve by adding some herbs or other different flavors.

(2) Mayonnaise: The color of this product was pale yellow and yellow in the products made from CF-2 and OF oil blends, respectively. The panelists preferred the color of the second product than the first one. They also noticed that the flavor of the last product was slightly rancid and its consistency was viscous and not semisolid. Therefore, the overall acceptability of this product was slightly like. On the other side, the second product prepared from OF oil blend was with unrancid flavor, semisolid consistency and a moderate like acceptance.

The above results showed that the sensory properties of the fatty omega products were depended on the fatty acids composition and the oxidation stability of the oil blend. Mayonnaise product made from oil with high level of unsaturated fatty acids was more susceptible for oxidation and had a thin consistency.

(3) Fresh sauerkraut salad: The results of the evaluation of the sensory properties of this product showed that the panelists did not detect differences in the sensory properties between the products containing CF-2 and OF oil blends. Both products had a red-purple color, crisp texture, and unrancid flavor. Therefore the overall acceptability of this product was very like as described by panelists. This product considers low calories, rich in dietary fibers and phytosterols (Simopoulos and Robinson, 1999).

(4) Carrot cake: This product contained carrot plus n-3 fatty acids from both CF-2 and/or OF oil blend. The results of the sensory evaluation indicated that the crust color of the carrot cakes prepared either by using CF-2 and OF oil blends was dark brown comparing with that made with butter and free from carrot, golden brown in color. Also the crumb color, porous distribution, porous structure, crumb texture and chewiness of the products containing the two oil blends were descried by panelists as yellow, fairly uniform, moderate, slightly soft and good, respectively. Such properties were less than those recorded for the control cake free from carrot and made of butter. The control cake had a white crumb color, uniform porous distribution; fine or thin cells porous structure, soft moist crumb texture and an excellent chewiness. The taste of the three cakes was accepted by panelists, the degree of the taste acceptability was excellent in both control cake and that made by CF-2 oil blend and good for the other product.

Conclusion

The biological effect of some vegetable oil blends rich in omega-3 fatty acids on rats showed that both source and composition of oil in the diet had an effect on the weight gain, liver weight, triglycerides, total cholesterol, LDL-cholesterol of the plasma and histopathology of the liver tissues of the rats. The results showed that the presence of n-3 fatty acids in the diets reduced the changes in the liver tissues. The shelf life of microcapsules containing oil blends to use as food supplement product can be recommended to be less than 6 months. Addition of antioxidants to such oil blends can extend their shelf life. The influence of such additives on biological effect of oil blends needs to examine. The capsules provide a convenient way to cover the personal need from the right fat and its optimum level of the required essential fatty acids.

The sensory properties of the prepared fatty omega products were acceptable by panelists.

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