Incorporation of flaxseed oil in producing functional ice milk and evaluate its health effect in rats

M. A. Ali * and I. M. Mahrous

Dairy Department, Faculty of Agriculture, Al Azhar University, Nasr City, Cairo, Egypt

* Corresponding author E-mail: DR.Mansourabdou@azhar.edu.eg (M. Ali).

ABSTRACT

Ice cream is a very popular dairy product throughout the world. The target of the present study is to produce a functional ice milk product rich in unsaturated fatty acids content by using different concentrations of flaxseed oil. Therefore, three different mixes of ice milk were prepared by substituting 25, 50 and 75% of milk fat (3%) with flaxseed oil. Results of fatty acid profiles of resultant plain and functional ice milk showed that the highest concentration of saturated fatty acids was present in control sample, being 63.72%, while the lowest figure was detected in F3 treatment (75% flaxseed oil substitute of milk fat), actually 21.1%. In contrast, control sample possessed the lowest value of unsaturated fatty acids, being 34.96%, while the highest concentration of unsaturated fatty acids was found in treatment F3 (77.58%). Moreover, omega-3 fatty acid content increased from 1.87% in control sample versus to 11.97% for F3 treatment. Continuously, omega-6/omega-3 ratio decreased significantly from 2.49 of control sample to 0.27 for F3 treatment. This decreases in omega-6/omega-3 ratio increased by increasing flaxseed oil substitution % in the ice milk mixes. However, decreasing omega-6 intake and increasing omega-3 are essential to improve health, prevention and management of chronic disease. Moreover, rats fed on functional ice milk reduced the levels of atherogenic indexes 1, 2 and LDL/HDL as compared to those fed on control ice milk (CII). In conclusion, health promoting could be provoked when flaxseed oil incorporated in making ice milk for up to 75% substitution of milk fat.

Keywords: Functional ice milk; Omega-3; Unsaturated fatty acids.

INTRODUCTION

Ice cream is a very popular dairy product throughout the world. Although people are fond of ice cream, they try to avoid its consumption due to presence of high percentage. The fatty acid composition of milk is characterized by high proportion of saturated fatty acids (60 – 70%) appreciable amount of mono unsaturated fatty acids (25 – 35%) and small amount of polyunsaturated fatty acids (about 4%) and omega3 fatty acid content in milk fat is less than 0.75%. Milk fat also contains 0.25 – 0.38% cholesterol (Jensen, 2002; Mathur *et al.,* 2005).

High fat intake is related to the development of nutrition related diseases, such as obesity, cardiovascular disease and some form of cancer. Therefore, nutritionist's advice that dietary fat should contribute less than 30% of total energy intake.

In recent years, consumption of reduced or low-fat dairy product such as ice milk has been increasing popular among health-conscientious consumers (Thayer, 1992).

Ice–milk is considered as one of ice cream related products and generally classified as frozen dessert which contain 2-7% fat and not less than 11% total milk solids.

The global guidelines suggest the desirability of deceasing consumption of total and saturated fat and cholesterol by substituting butterfat with vegetable oils sash as sunflower oil, flaxseed oil (USDAH, 2000).

On the other hand, functional foods may be defined as foods or dietary components that may provide significant health benefits in addition to basic nutrition. The term essential fatty acids (EFA) refers to those polyunsaturated fatty acids (PUFA) that must be provided by foods because these cannot be synthesized in the body yet are necessary for health. There are two families of EFA, omega-3 (ω-3) and omega-6 (ω-6). (Marszalek and Lodish, 2005; Kour *et al.,* 2014).

Flaxseed *(Linum usitatissimum)* also known as linseed, contains 32 – 45% of it mass as oil, of which 51 – 55% is alpha-linolenic-acid "ALA" (18.3, n-3 Omega-3 fatty acid), 12.7% linoleic acid "LA" (18.2, n-6 Omega-6 fatty acid), as well as being a good source of dietary fibers and lignans (Braun and Cohen, 2007). Human consumption of omega-3 fatty acid is proven to decrease the incidence of cardiovascular disease, reduce inflammation and prevent certain chronic disease such as diabetes, hypertension, cancer, autoimmune disease and arthritis (Kris-Etherton *et al*., 2003; Larsson *et al*., 2004). Omega-3 fatty acids have anti-atherogenic of coronary artery, anti-proliferative of tumor, reduce low-density lipoprotein (LDL), lower serum triglycerides and other health beneficial effects (Braun and Cohen, 2007).

Therefore, the aim of this study is to produce a Functional ice milk, rich in unsaturated fatty acids content especially omega-3 and to evaluate the biological effect of consumption ice milk containing different levels of flaxseed oil on lipid profile of albino rats.

MATERIALS AND METHODS

Characterization

Milk and Cream

Fresh cow's milk (3% fat) was obtained from the herd of Faculty of Agriculture, Cairo Univ., Giza, Egypt. The Fresh milk was separated to obtain fresh cream (40% fat).

Skim milk powder

Skim milk powder (34% protein, 1.23% fat and 4% moisture) was purchased from The Nile Commercial CO. Cairo, Egypt.

Flaxseed oil

Cold pressed Flaxseed oil was obtained from Extract and Press Oil Unit, National Research Center, Dokki, Giza, Egypt.

Fruits

Mango fruit was purchased from local markets, Cairo, Egypt.

Preparation of fruit pulp

Mango fruit was sorted, cleaned, washed, processed and homogenized by ultra truxx for 5 min until obtaining a homogenous mass, then treated in steam jacketed vessel at 80 ˚C for 1 min., pulp was extracted by means of a fine pulper in order to get homogenous textured pulp and then cooled at room temperature. The citric acid 0.08 %, was added to the pulp. The resultant pulp was filled and placed into 200 mL sterilized vessels glass can, which was then stored in a frozen condition at 18±2°C for later use.

Sugar Cane

Commercial grade granulated sugar cane obtained from Sugar and Integrated Industries CO., Giza, Egypt.

Stabilizers

Indian carboxyl methyl cellulose (CMC), high viscosity with minimum assay 95%, was purchased from local markets, Cairo, Egypt.

Emulsifiers

Mono and Diglycerides (E 241, E 242) were obtained from E.W.A. CO., Sheraton, Cairo, Egypt.

Maltodextrin and Vanillin

Maltodextrin and Vanillin essence were purchased from local markets. Cairo, Egypt.

Animals (Albino rats)

Twenty-five mature male albino rats were used in the present study with mean body weights of 130±5gm., the rats were obtained from Animal house, National Research Center, Cairo, Egypt.

Basel diet

The chemical composition of basal or control diet was as follows: starch, 67%; raw protein, 23%; raw fat, 6.40%; and fibers, 3.60%.

Methods

Preparation of ice milk mixes

The ice milk mix consisted of 3% fat, 12% SNF, 15% sugar, 0.2% CMC, 0.3% emulsifiers and 0.06% vanilla essence and fruit pulp were added into the chilled mixes extract served as a control.

Ice milk containing flaxseed oil was prepared by substituting 25, 50 and 75 % of milk fat in ice milk mixes with flaxseed oil respectively as follows:

(C) 3:0, (F1) 2.25:0.75, (F2) 1.5:1.5 and (F3) 0.75:2.25.

Mix processing

Ice milk mixes manufacture of according to Marshall and Arbuckle (1996).

Determination and identification of fatty acids

The fatty acids profile of lipid extracted from prepared ice milk mixes were determined by gas chromatography (GC) with flame ionization detector (FID) according to A.O.A.C. (2011).

Feeding Experiment

Rats were randomly and equally divided into five groups, five rats for each group. The animals were housed individually in standard polypropylene cages at room temperature (25±2°C) and relative humidity (about 55%) for 28 days. The rats were acclimatized on basal diet for one week before starting the experiment.

After an adaptation period, the first group was fed on basal diet (70 g. for each rat/day) and served as control (C1), while the other second group were fed on basal diet plus ice milk (3% milk fat) and served as control II (CII). the rats three groups were fed as follows:

Group (A) were fed on basil diet containing ice milk (F1).

Group (B) were fed on basil diet containing ice milk (F2).

Group (C) were fed on basil diet containing ice milk (F3).

All rats were weighed at the beginning and the end of the experimental period.

Blood Samples

At the beginning of the experiment and after adaptation period blood samples were drawn from the retrobulber nenous plexus of each rate through a capillary glass tubes and left to clot at room temperature to obtain a clear serum. At the end of the experiment, the rats were killed by withdrawn blood from the abdominal aorta under light diethyl ether anesthesia. Blood were collected in heparin added tubes.

Determination of total cholesterol content

The total cholesterol concentration was determined in the serum using Bio Med diagnostic kit:

$$\text{Cholesterol (mg/dL)} = \frac{\textbf{A of the tested sample}}{\textbf{A of the standard solution}} \times 200$$

Where: A= Absorbance at 546 nm.

Determination of high-density lipoproteins (HDL)-cholesterol content

HDL-cholesterol was determined in serum by using Bio Med diagnostic kit. and the following equation:

Concentration of HDL-cholesterol (mg/dL) in supernatant = A sample × 325

Where: A= Absorbance at 546 nm.

Determination of low-density lipoproteins (LDL)-cholesterol contents

The LDL-cholesterol was estimated according to the formula of Beena and Prasad (1997) as follows:

$$\text{LDL-cholesterol (mg/dL)} = \text{Total cholesterol} - \left(\textbf{HDL} + \frac{\textbf{triglycerides}}{\textbf{5}}\right).$$

Determination of very low-density lipoproteins (VLDL)-cholesterol contents

The VLDL-cholesterol level was estimated according to the formula of Fridewald *et al*. (1972) as follows:

VLDL-cholesterol (mg/dL) = Triglycerides/5

Determination of Triglycerides

The triglycerides content was determined in the serum by using Bio Med diagnostic kit and the following equation:

$$\text{Triglycerides (mg/dL)} = \frac{\textbf{A of the tested sample}}{\textbf{A of the standard solution}} \times 200.$$

Where: A= Absorbance at 500-550 nm.

Calculation of atherogenic index

The atherogenic index were calculated according to Zommara *et al*. (2006), as follows:

atherogenic index 1: LDL/Total cholesterol

atherogenic index 2: (Total cholesterol – HDL) / HDL

Biological Evolution of rat diets

Biological evolution of the different diets was carried out by determination of body weight gain, food efficiency ratio and growth rate, g / day according to Carthew *et al*. (2001) by using following formulas:

Body weight gain = final weight – initial weight

$$\text{Food efficiency ratio} = \frac{\textbf{Growth rate}, \frac{\textbf{g}}{\textbf{day}}}{\textbf{Food intake}, \frac{\textbf{g}}{\textbf{day}}}$$

Growth rate, g/day = $\frac{\text{Body weight gain, (g)}}{\text{Experimental period long, (days)}}$

Statistical analysis

The data obtained from three replicates were analyzed by (ANOVA) using the SPSS statistical package program, and differences among the means were compared using the Duncan's Multiple Range test (SPSS, 1998). A significant level of 0.05 was chosen.

RESULT AND DISCUSSION

The present study aims to evaluate the suitability of flaxseed oil as healthy oil in formulation of functional ice milk to decrease the nutritional gap of ω-3 fatty acids in vegetarian diet, which can be fulfilled by incorporation of omega-3 fatty acids rich sources such as flaxseed oil.

Table 1. Fatty acids profile of pure flaxseed oil**.**

Fatty acids	Common name	% Content
(C16:0)	Palmitic acid	5.0
(C18:0)	Stearic acid	3.9
(C18:1) ω9	Oleic acid	18.91
(C18:2) ω6	Linoleic acid	14.83
(C18:3) ω3	α-Linolenic acid	55.93
(C18:1) ω7	Vaccenic acid	1.0
C18:4 ω3	Stearidonic acid	0.14
C20:0	Arachidic acid	0.12
C20:1	Gadoleic acid	0.1
Non Identified Fatty acids		0.07
Total Saturated Fatty Acids (TSFA)		9.02
Total Unsaturated Fatty Acids (TUSFA)		90.91
Omega6/Omega3 Ratio		0.26

Flaxseed oil was extracted by the cold pressing method containing 8 fatty acids (3 saturated and 5 unsaturated) were detected as shown in Table 1. Also, it had high content in TUSFAs 90.02%of total fatty acids. Concerning C18:3 fatty acid content, it composed about 55.93% of total fatty acids, while linoleic and oleic fatty acids were 14.38 and 18.91% respectively.

These results are agreement with those reported by El-Waseif (2012) and Popa *et al*. (2012). On the other hand, total saturated fatty acids content in flaxseed oil was 9.02% of total fatty acids. Plametic and stearic acids were the main saturated fatty acids in flaxseed oil with lower content actually 5 and 3.9% of total fatty acids. Similar findings were previously reported by Oomah (2001) and Daun *et al*. (2003)**.**

Table 2. Formulation of plain and functional ice milk mixes.

Components / Treatments	Control	F1	F2	F3
Fresh Skim Milk	54.74	55.86	57.00	58.12
Skim Milk Powder	7.20	7.20	7.20	7.20
Cream40%	7.50	5.63	3.75	1.87
Flaxseed Oil	0.00	0.75	1.50	2.25
Sugar	15.00	15.00	15.00	15.00
Fruit's pulp*	15.00	15.00	15.00	15.00
Stabilizers	0.20	0.20	0.20	0.20
Emulsifiers	0.30	0.30	0.30	0.30
Vanillin Essence	0.06	0.06	0.06	0.06
Total	100	100	100	100

Moreover, the obtained data from the same table showed that polyunsaturated fatty acid omega6/omega3 ratio was 0.26%this ratio is the optimum ratio between these acids for health. These results are in agreement with Kim *et al*. (2007); Simopoulos (2008) and Russo (2009).

In order to produce functional ice milk, three different mixes of ice milk prepared by substituting 25, 50 and 75% of milk fat with flaxseed oil in order to modify the fatty acids profile of the resultant ice milk and enrich its level of omega-3, which make the resultant ice milk,15% of mango pulp as natural flavor and 1.5% maltodextrin were added.

Table 3. Saturated fatty acids in plain and functional ice milk treatments.

Treatments / Fatty acids	Common name	C	F1	F2	F3
C4:0	Butyric acid	1.44	1.39	1.26	0.91
C6:0	Caproic acid	1.23	0.98	0.53	0.29
C8:0	Caprylic acid	0.73	0.60	0.37	0.19
C10:0	Capric acid	1.67	1.24	0.81	0.35
C12:0	Lauric acid	1.97	1.52	0.95	0.45
C14:0	Myristic acid	8.91	6.65	4.06	1.90
C15:0	Pentadecylic acid	2.76	2.01	1.29	0.51
C16:0	Palmitic acid	30.93	24.11	17.39	10.36
C17:0	Margaric acid	2.46	2.24	1.10	0.32
C18:0	Stearic acid	11.31	9.73	7.55	5.63
C20:0	Arachidic acid	0.31	0.27	0.24	0.19
Total Saturated Fatty Acids (TSFAs)		65.32	41.7	35.95	21.11

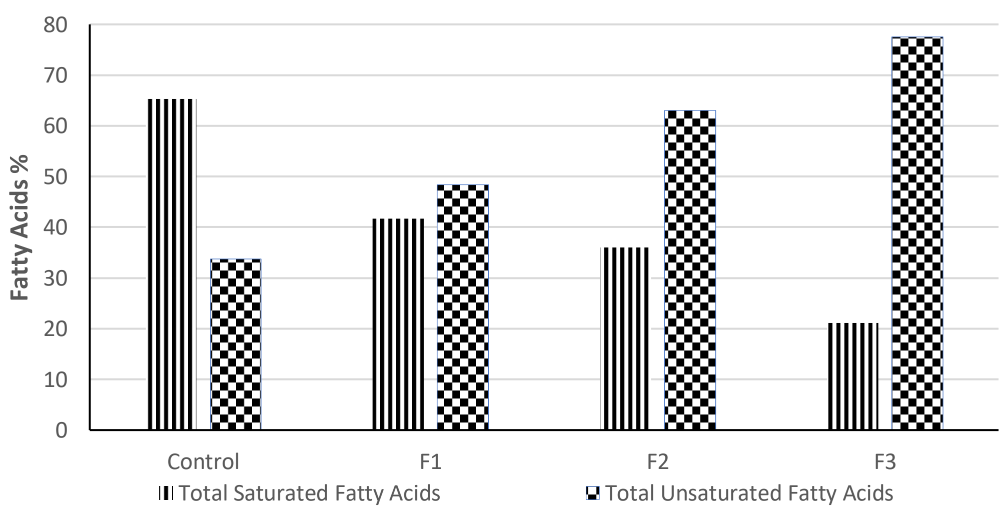

Figure 1. Saturated and unsaturated fatty acids in ice milk treatments.

As seen from Table 2 functional ice milk ingredients were standardized to give 12% milk solids not fat, 15% sugar, 0.2 %C.M.C, 0.3%, emulsifiers, 0.06 % vanillin extract and 15% mango's pulp as flavoring ingredients.

Fatty acids profile of resultant plain and functional ice milk was carried out gas chromatography analysis and the obtained data are recorded in Tables 3 and 4 and graphically plotted in Figure 1.

Data in Table 3 and figure. 1 showed that 11 saturated fatty acids were detected in all samples. Also, the total saturated fatty acids were 63.72, 50.74, 53.55 and 21.1% for control, F1, F2 and F3 treatment respectively. However, the content of saturated fatty acids was decreased by increasing level of flaxseed oil into ice milk mixes. This may be attributed to higher total unsaturated fatty acids content in flaxseed oil (90.91%). These results are in agreement with those reported by Goh *et al*. (2006); Lim *et al*. (2010) and El-Waseif (2012).

Furthermore, the data presented in table 4 and figure.1 showed that ice milk treatment content of unsaturated fatty acids ranged from 33.76 to 77.52% of total fatty acids for control to F3 treatment, respectively.

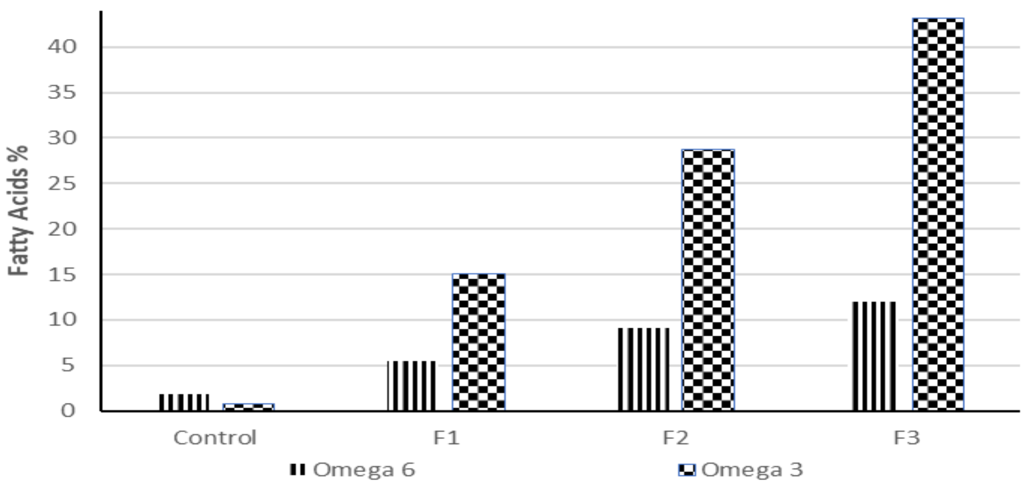

Figure 2. omega 6 and omega 3 in different ice milk treatments**.**

However, incorporation of flaxseed oil into ice milk mixes during formulation extracted marked increase in omega-3fatty acid (ALA) content from 0.75% for control to 43.18% for F3 treatment. Moreover, the omega-6 content in ice milk treatment increased from 1.87% in control to 11.947% for F3 treatment, of total fatty acids, while the oleic acid content was significantly decreased with increase of incorporation of flaxseed oil into ice milk treatments, it was ranged from 22.8 to 19.86% of the total fatty acids for control to F3 treatment, respectively. This may be due to the decrease in whipping cream quantity. Since whipping cream was not a good source of linoleic and linolenic acids, theincorporation of flaxseed oil in ice milk had boosted up the quantity of both fatty acids. These results are in agreement with Goh *et al*. (2006); Lim *et al*. (2010) and Danthine (2012).

The data from Table 4 and figure 2, showed that poly unsaturated fatty acids omega-6/omega-3 ratio was 2.49 for control sample, this ratio decreased significantly to be in F3 treatment 0.27, this decreased in omega 6/omega3 ratio increased by increasing flaxseed oil concentration into ice milk. The importance of incorporation of flaxseed oil into ice milk, not only for its high content from unsaturated fatty acids, but also for improving various characteristics such as providing a good balance for omega6/omega3 ratio.

Table 4. Unsaturated fatty acids in plain and functional ice milk treatments.

Fatty acids / Treatments	Common name	C	F1	F2	F3
C14:1	Myristoleic acid				
C16:1ω7	Palmitoleic acid	1.58	1.18	0.81	0.4
C16:3ω4	Hexagonic acid	0.45	0.30	0.25	-
C18:1ω9	Oleic acid	22.81	21.93	21.14	19.86
C18:1ω7	Vaccenic acid	3.18	2.54	2.05	1.52
C18:1ω5	Octadecosaenoic acid	0.8	0.46	0.37	0.24
C20:1ω11	Gadoleic acid	0.42	0.24	-	-
Total Mono Unsaturated Fatty Acids (TMUSFAs)		29.42	26.65	24.62	22.17
C18:2ω5		0.29	0.24	-	-
C18:2ω6	Linoleic acid	1.87	5.43	9.15	11.97
C18:2ω4		0.39	0.29	-	-
C18:3ω3	α-Linolenic acid	0.75	15.09	28.74	43.18
C18:4ω3	Octadecatetraenoicacid	0.82	0.68	0.53	0.2
Total Poly Unsaturated Fatty Acids (TPUSFAs)		4.34	21.73	38.42	55.35
Non identified fatty acids		0.58	0.56	0.85	0.64
Total Unsaturated Fatty Acids (TUFAs)		33.76	48.38	63.04	77.52
ω6/ω3 ratio		2.49	0.36	0.31	0.27

However, decreasing omega 6 intake while increasing the omega-3 is essential to improve health, prevention and management of chronic disease by reducing the risk of cardiovascular disease (CVD) obesity, diabetes, inflammation and several neurological diseases. (Choo *et al*. 2007; Candela *et al*. 2011 and Tou *et al*. 2011).

It was evident, as the data in Table 4 that F3 treatment is the optimal treatment in omega6/ omega 3 ratio as compared to the corresponding ratio in flaxseed oil, being 0.27 and 0.26 for F3 and flaxseed oil, respectively. These results are in agreement with those mentioned by Simopoulos (2002); Kim *et al*. (2007) and Burghart *et al*. (2010).

Generally, from the obtained foregoing result, the current study recommended that flaxseed oil can be incorporate in making functional ice milk up to 75% substitution on milk fat and represent an excellent source of omega-3 to fulfill the nutritional gap of ω-3 fatty acids and providing a good balance for omega6/omega3 ratio.

Adding both 15% mango`s pulp and maltodextrin at ratio 1.5% to make advantages of multiple nutritional and health benefits for flaxseed oil as functional ingredient in ice milk product.

Hypercholesterolaemic is major risk factor associated with coronary heart diseases; therefore, keeping blood cholesterol at a desirable level is one of the major preventive strategies for these diseases. The diet containing polyunsaturated fatty acids (PUFAs), can play an important role in the control of lipid profile and cholesterol homeostasis (Cintra *et al*., 2006 and Vijaimohan *et al*., 2006).

The ingestion of PUFAs (N-3 and N-6) is related to the lowering of the incidence of heart disease by decreasing cholesterol and triacylglycerol plasmatic levels (Amate & Ramirez, 2001, Gebauer *et al*., 2006 and Bernacchia *et al*., 2014).

Therefore, the objective of the current study was to evaluate weather the incorporation of functional ice milk with different doses of flaxseed oil to the normal diet for albino rats for 28 days, can produce beneficial effects on lipid profile and consequently on the prevention of atherosclerosis. Results obtained for growth parameters were. plotted in Table 5. From these dates, it could be seen that no noticeable differences in initial body weight between all tested groups. However, rats fed on different ice milk treatments gained more body weight at the end of the experiment as compared with dry control group.

In addition, no significant differences were found among dietary groups in food intake, while the average of body weight gain for rats fed on ice milk treatments were higher than rats fed on dry diet, the body weight gain values were 39 and 67g. for control dry diet and control ice milk respectively. On the other hand, there is no variation in body weight gain for rats fed on ice milk treatments. This finding suggests that type of diet significantly affect the growth parameters of rats. The same trend of results was previously reported by Mohamed *et al*., (2011); Tou *et al*., (2011) and Boulbaroud *et al*. (2012).

Table 5. Growth parameters of rats fed on ice milk containing different concentrations of flaxseed oil for 28 days.

Treatments / Parameters	Control dry diet	Control 3%fat	F1	F2	F3
Initial body weight (g)	133 A	133 A	135 A	132 A	133 A
Final body weight (g)	172 B	200 A	198 A	196 A	197 A
% change to normal control	-----	16.27	15.11	13.95	14.53
Food intake (g/day)	14 B	16 A	16 A	16 A	16 A
Body weight gain (g)	39 B	67 A	63 AB	64 AB	64 AB
Body weight gain (%)	29.3 C	50.38 A	46.66 B	48.48 AB	48.12 AB
Growth rate, (g/day)	1.39 B	2.39 A	2.25 AB	2.28 AB	2.28 AB
Food efficiency	0.10 B	0.15 A	0.14 AB	0. 14 AB	0.14 AB

Continuously, the present results declared that there were noticeable variations between growth rate (g, body weight gain / feeding period) or food efficiency (growth rate (g/day)/ food intake) among different ice milk treatments. Whereas, there were a significant increase growth rate between ice milk treatments and the control dry diet, growth rate recorded for control dry diet 1.39 whereas it ranged from 2.39 - 2.25 g/day for control ice milk to F3 treatments respectively. The same trend of results was in food efficiency, the values were 0.10 for control dry diet, while it ranged from 0.15 - 0.14 with non-considerable variations between all ice milk treatments. These variations in results may be attributed to the nature of diet which were without cholesterol supplementation, these results in agreement with those reported by Vijaimohan (2006); El- Waseif (2008) and Mohamed *et al*., (2011).

The effect of feeding functional ice milk on lipid profile was carried out and results obtained were summarized in tables and.it could be observed that no significant differences in initial serum total cholesterol between all tested groups. However, at the end of the experiment, rats fed on control ice milk ranked the highest value, actually 101.92 mg/dL, while lower figure, being 91.31 mg/dL, was recorded for control dry diet.

On the other hand, flaxseed oil was effective in reducing serum cholesterol, it could be noticed that the total cholesterol serum in groups fed on different ice milk treatments reduced gradually as compared with control ice milk, the reduction % ranged from (-) 16.24 to (-) 35.76% for F1-F3 treatments respectively. These reductions in total cholesterol may be due to the presence of flaxseed oil into ice milk treatments.

These results are in agreement with those reported by Lucas *et al*., (2004); Boulbaroud *et al*., (2012) and Soltan (2012).

In this respect, Moghadasian (2008), Brenna *et al*., (2009) and Tzang *et al*., (2009) reported that lowering total cholesterol by flaxseed oil may be due to increase omega-3 long chain polyunsaturated fatty acid in rats by a conversion of ALA in flaxseed oil to EPA and DHA. EPA+DHA has been associated with reduced risk of coronary heart disease.

Table 6. Levels of serum total cholesterol and HDL-cholesterol in rats fed on ice milk containing different concentrations of flaxseed oil for 28 days.

Treatments / Parameters	Control dry diet	Control 3%fat	F1	F2	F3
Initial total cholesterol (mg/dL)	85.34 A	85.20 A	85.10 A	85.65 A	86.10 A
Final total cholesterol (mg/dL)	91.31 B	101.92 A	85.37 B	70.49 C	65.47 D
% Change to normal control	------	(+) 11.62	(-) 6.51	(-) 22.80	(-) 28.30
Initial HDL-cholesterol (mg/dL)	47.75 A	47.30 A	48.91 A	48.20 A	46.89 A
Final HDL-cholesterol (mg/dL)	50.22 A	50.10 A	43.81 B	37.83 C	36.43 C
% Change to normal control	-----	(-) 0.24	(-) 12.76	(-) 24.67	(-) 27.50

*Values with different small letters in the same row are significantly different ($p<0.05$).
HDL: high-density lipoprotein (+): increase (-): decrease

The effect of the experimental diets on the levels of high-density lipoprotein (HDL) cholesterol are presented in Table (6). It was obvious from obtained data that there was a slight decrease in HDL- cholesterol levels in blood serum of rats fed on control ice milk as compared with those fed on control dry diet, actually 0.24%.

However, comparing the obtained results of rats fed on ice milk containing flaxseed oil with corresponding figure of control ice milk treatment, showed variable decreasing in HDL-cholesterol values ranged from `12.55% to 27.29%. In addition, it could be noticed that the decrease % of HDL-cholesterol were increased as flaxseed oil substitution% increased.

These significant effects may be attributed to the total serum cholesterol which decreases with the increase of flaxseed oil concentration into ice milk treatments. These results are in agreement with those reported by Morise *et al*., (2004); Boulbaroud *et al*., (2012); EL-Sahar *et al*., (2014) and Aly-Aldin *et al*., (2015).

Triglycerides in all tested groups showed variable levels at the end of feeding experiment as compared to initial triglycerides.

The obtained results shown in Table (7) revealed that addition of fresh cow's milk (3% fat) increased serum triglycerides by 27.48% as compared to dry diet (control I), it being 92.9 mg/dL. On the other hand, supplementation of diets with different ice milk treatments after 4 weeks of feeding period resulted in noticeable decreases in serum triglycerides in treatments F1 to F3, the reduction values varied from 19.40 to 46.07 % for F1 to F3 treatments respectively as compared to control ice milk. This decrease in triglycerides content may be attributed to the presence of flaxseed oil, which may affect the triglycerides levels in serum.

These results are in agreement with those mentioned by Riediger *et al*., (2008); EL-Sahar & Abed EL- Rahman (2014) and Khan& Makki (2017).

Since a high blood LDL–cholesterol is associated with increased risk of atherosclerosis and cardiovascular disease, any product that lowers this level is of potential value. Therefore, the main target of this part of investigation was to gain some information concerning the effect of feeding different types of ice milk treatments on low–density lipoprotein (LDL) cholesterol.

Table 7. Levels of serum triglycerides, LDL-cholesterol and VLDL-cholesterol in rats fed on ice milk containing different concentrations of flaxseed oil.

Parameters / Treatments	Control Dry diet	Control 3%fat	F1	F2	F3
Initial Triglycerides (mg/dL)	68.22 A	66.99 AB	67.10 AB	68.95 A	68.30 A
Final Triglycerides (mg/dL)	72.87 B	92.90 A	73.85 B	57.10 C	50.10 D
% change to normal control	-----	(+)27.48	(-)19.4	(-)38.54	(-)46.07
Initial LDL-cholesterol (mg/dL)	23.95 B	24.50 AB	23.77 B	23.66 B	25.55 A
Final LDL-cholesterol (mg/dL)	26.52 B	33.24 A	26.79 B	21.24 C	19.02 D
% change to normal control	-----	(+) 25.34	(+) 19.40	(-) 36.10	(-) 42.77
Initial VLDL-cholesterol (mg/dL)	13.64 A	13.40 A	13.42 A	13.79 A	13.66 A
Final VLDL-cholesterol (mg/dL)	14.57 B	18.58 A	14.77 B	11.42 C	10.02 D
% change to normal control	----	(+) 27.52	(-) 20.50	(-) 38.53	(-) 46.07

*Values with different small letters in the same raw are significantly different ($p<0.05$).

LDL: low-density lipoprotein VLDL: very low-density lipoprotein (+): increase (-): decrease

As seen in Table (7) that, there is no significant differences in initial LDL cholesterol between all tested groups, whereas at the end of the 4- weeks experimental period there was high significant difference in LDL–cholesterol levels between different treatments, the final levels of LDL cholesterol in serum of rats fed on control ice milk increased by 25.34% for control ice milk as compared to control dry diet. In contrast, the levels of LDL – cholesterol in rats fed on ice milk containing different doses of flaxseed oil were noticeably decreased with the increasing of flaxseed oil into ice milk, the reduction values ranged from -19.4 to - 42.77% for F1 to F3 treatment respectively, this reduction may be attributed to the presence of flaxseed oil, these results are in agreement with Morise *et al*., (2004); Vijaimohan *et al*., (2006); Boulbaroud *et al*., (2012) and Kawakami *et al*., (2015).

VLDL cholesterol is an independent predictor of CHD risk and overall appears to be a better predictor of CHD risk than LDL cholesterol. VLDL concentration paralleled that of TG and was therefore dramatically higher in the rats fed on the control ice milk diet than in the other groups (Liu *et al*., 2006).

As shown from the same data it could be noticed that no significant differences in initial VLDL levels between all tested rats. However, VLDL levels in rats fed on control ice milk treatment at the end of the experiment increased by 27.52 % as compared with rats fed on control dry diet. On the other hand, the rats fed on ice milk treatments which contain different doses of flaxseed oil the VLDL levels markedly decreased, the reduction values ranged from - 20.50 to -46.07% for F1 to F3 treatment respectively as compared with control ice milk treatment. These results in agreement with previous results of Morise *et al*., (2004); Vijaimohan *et al*., (2006) and Fadlalla *et al*., (2013).

The decrease in serum triacylglycerol, LDL and VLDL of F1 to F3 treatments can be explained by Strolien *et al*., (2007) the authors suggested that the decrease in triglyceride levels attributed to the lowering effect of omega-3 fatty acids has been mainly ascribed to reduce hepatic synthetic of VLDL. Omega-3 fatty acid suppressed hepatic lipogenesis and reduced circulating TG level.

As a matter of fact, the atherogenic index is an important tool for analyzing the results of clinical trials. The association of TGs and HDL-C in this simple ratio theoretically reflects the balance between risk and protective lipoprotein forces, and both TGs and HDL-C are widely measured and available (Dobiášová 2004).

Table 8. Levels of atherogenic indices in rats fed on ice milk containing different concentrations of flaxseed oil for 28 days.

Treatments / Parameters	Control dry diet	control 3%fat	F1	F2	F3
Final HDL/Total cholesterol	0.550 A	0.492 B	0.513 AB	0.537 A	0.556 A
Atherogenic index 1	0.290 C	0.326 A	0.314 A	0.301 B	0.290 C
Atherogenic index 2	0.818 D	1.034 A	0.949 B	0.863 C	0.797 D
% LDL/HDL ratio	52.81 D	66.35 A	61.15 B	56.15 C	52.21 D

*Values with different caoital letters in the same row are significantly different ($p<0.05$).
atherogenic index 1: LDL/Total cholesterol (+): increase
atherogenic index 2: (Total cholesterol-HDL)/HDL (-): decrease.

Atherogenic index in recent years has started to gain importance as an indicator of atherosclerosis. (Söğüt *et al*., 2006).

Therefore, the atherogenic indices and the ratio between LDL or HDL – cholesterol and total cholesterol, in addition to LDL /HDL ratio were calculated and data obtained illustrated in Table 8.

As shown from these results that supplementation of cow's milk to dry diet, led to increasing values in atherogenic indices 1 and 2, in addition to LDL/HDL ratio, actually it recorded 0.326, 1.034 and 0.663 respectively.

Moreover, as could be gathered from the same table that only rats fed on ice milk with flaxseed oil doses the levels of atherogenic indexes 1, 2 and LDL/HDL ratio decreased by a mean values ranged from 0.314 -0.291, 0.949- 0.797 and 61.15- 52.21 respectively as compared to those fed on control ice milk. However, because these parameters were calculated from the values of total cholesterol, HDL-cholesterol and LDL-cholesterol, which were pronounced affected by dietary ice milk incorporated flaxseed oil, thus, the reduction in atherogenic indices and LDL/HDL ratio would be expected.

Similar trend of results was previously reported by Dupasquier *et al*., (2007) and Gruenfelder (2014) who demonstrated that dietary flaxseed can inhibit atherosclerosis through a reduction of circulating cholesterol levels and, at a cellular level, via anti-proliferative and anti-inflammatory actions.

CONCLUSION

In conclusion, from the forgoing results, it could be stated that rats fed on ice milk congaing flaxseed oil showed pronounced hypolipidemic effect, as reduced serum cholesterol, LDL-cholesterol and serum triglyceride. Moreover, it could be gathered from data obtained that incorporation of flaxseed oil, in different concentration reduced markedly the levels of atherogenic indexes 1,2 and LDL/HDL ratio in treatments F1 to F3 as compared with the corresponding figure in control sample.

REFERENCES

Aly-Aldin, M.M., Mansour, E.H., Rahma, E.H., Abo El-Fath, A.E.E., El-Bedawey, F.A., El-Habashy, M.M., 2015. Protective role of flaxseed oil on hypercholesterolemic rats. Biolife, 3 (4), 794-801.

Amate, L., Gil, A., Ramírez, M., 2001. Dietary long-chain polyunsaturated fatty acids from different sources affect fat and fatty acid excretions in rats. J. Nutr., 131(12), 3216-3221.

A.O.A.C., 2011. Official Methods of Analysis of AOAC International. 18thed Published by the AOAC. International (revised edition), Gaithersburg, Maryland, U.S.A.

Beena, A., Prasad, B., 1997. Effect of yoghurt and bifidus yoghurt fortified with skim milk powder, condensed whey and lactose–hydrolyzed condensed whey on serum cholesterol and triacylglycerols level on rats. J. Dairy Res. 64 (3),453-457.

Bernacchia, R., Preti, R., Vinci, G., 2014. Chemical composition and health benefits of flaxseed. Austin J. Nutr. Food Sci., 2 (8), 1045.

Boulbaroud, S., El-Hessni, A., Azzaoui, F.Z., Mesfioui, A., 2012. Sesame seed oil and flaxseed oil affect plasma lipid levels and biomarkers of bone metabolism in ovariectomized Wistar rats. Biol. Med., 4 (3), 102.

Braun, L., Cohen, M., 2007. Herbs & Natural Supplements: An Evidence-Based Guide. Elsevier Australia, pp. 316-318.

Brenna, J.T., Salem, N., Sinclair, A.J., Cunnane, S.C., 2009. α-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. Prostag. Leukotr. Ess. Fatty Acids, 80 (2), 85-91.

Burghardt, P.R., Kemmerer, E.S., Buck, B.J., Osetek, A.J., Yan, C., Koch, L.G., Evans, S.J., 2010. Dietary n-3: n-6 fatty acid ratios differentially influence hormonal signature in a rodent model of metabolic syndrome relative to healthy controls. Nutr. Metabol., 7 (1), 53.

Candela, C.G., López, L.B., Kohen, V.L., 2011. Importance of a balanced omega 6/omega 3 ratio for the maintenance of health. Nutritional recommendations. Nutr. Hosp., 26 (2), 323-329.

Carthew, P., Baldrick, P., Hepburn, P.A., 2001. An assessment of carcinogenic potential of sheaoleine in the rat. Food Chem. Toxicol., 39, 807-818.

Choo, W.S., Birch, J., Dufour, J.P., 2007. Physicochemical and quality characteristics of cold-pressed flaxseed oils. J. Food Compos. Anal., 20 (3-4), 202-211.

Cintra, D.E., Costa, A.V., Maria do Carmo, G.P., Matta, S.L., Silva, M.T.C., Costa, N.M., 2006. Lipid profile of rats fed high-fat diets based on flaxseed, peanut, trout, or chicken skin. Nutrition, 22 (2), 197-205.

Danthine, S., 2012. Physicochemical and structural properties of compound dairy fat blends. international Food research. 48 (1), 187-195.

Daun, J.K., Barthet, V.J., Chornick, T.L., Duguid, S., 2003. Structure, composition, and variety development of flaxseed. In: Flaxseed in Human Nutrition, AOCS Publishing, pp. 6-40.

Dobiášová, M., 2004. Atherogenic index of plasma [log (triglycerides/ HDL cholesterol)]: theoretical and practical implications. Clin. Chem.; 50, 1113-1135.

Dupasquier, C.M., Dibrov, E., Kneesh, A.L., Cheung, P.K., Lee, K.G., Alexander, H.K., Pierce, G.N., 2007. Dietary flaxseed inhibits atherosclerosis in the LDL receptor-deficient mouse in part through antiproliferative and anti-inflammatory actions. Am. J. Physiol. Heart Circul. Physiol., 293 (4), 2394-2402.

EL-Sahar, E.G.E., Abed EL-Rahman, A.M.M., 2014. Study on the biological effect of use flaxseed oil as a source of fat on the biomarkers of experimental rats. J. Am. Sci., 10 (3), 116-123.

EL-Waseif, M.A.E., 2008. Chemical Study on Pure Linseed Oil. MSc. Thesis, Fac. Agric. Al-Azhar Univ., Cairo, Egypt.

El-Waseif, M.A.E., 2012. Producing some Therapeutic Oil and Fat Products. PhD Thesis. Fac. Agric. Al-Azhar Univ., Cairo, Egypt.

Fadlalla, E.A.S., AbdElal, Z.S., Seddik, A.A., 2013. Protective effect of olive, almond and flaxseed oil againstcarbon tetrachloride-induced hepatotoxicity in rat models. Afr. J. Biol. Sci., 6 (1), 243-259.

Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18 (6), 499-502.

Gebauer, S.K., Psota, T.L., Harris, W.S., Kris-Etherton, P.M., 2006. N-3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am. J. Clin. Nutr., 83 (suppl), 1526-1535.

Goh, K.K., Ye, A., Dale, N., 2006. Characterisation of ice cream containing flaxseed oil. Int. J. Food Sci. Technol., 41(8), 946-953.

Gruenfelder, C.A., 2014. Sensory evaluation of heart-healthy foods enriched with omega-3 fats from fish oil. PhD, College of Saint Elizabeth., N.J., USA.

Jensen, R.G., 2002. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci., 85 (2), 295-350.

Kaur, N., Chugh, V., Gupta, A.K., 2014. Essential fatty acids as functional components of foods, a review. J. Food Sci. Technol., 51 (10), 2289-2303.

Kawakami, Y., Yamanaka-Okumura, H., Naniwa-Kuroki, Y., Sakuma, M., Taketani, Y., Takeda, E., 2015. Flaxseed oil intake reduces serum small dense low-density lipoprotein concentrations in Japanese men: a randomized, double blind, crossover study. Nutr. J., 14 (1), 39.

Khan, S.A., Makki, A., 2017. Dietary changes with omega-3 fatty acids improves the blood lipid profile of Wistar Albino rats with hypercholesterolaemia. Int. J. Med. Res. Health Sci., 6 (3), 34-40.

Kim, S.C., Adesogan, A.T., Badinga, L., Staples, C.R., 2007. Effects of dietary n-6: n-3 fatty acid ratio on feed intake, digestibility, and fatty acid profiles of the ruminal contents, liver, and muscle of growing lambs. J. Anim. Sci., 85 (3), 706-716.

Kris-Etherton, P.M., Harris, W.S., Appel, L.J., 2003. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Arterioscler. Thromb. Vasc. Biol., 23 (2), e20-e30.

Larsson, S.C., Kumlin, M., Ingelman-Sundberg, M., Wolk, A., 2004. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am. J. Clin. Nutr., 79, 935-945.

Lim, C.W., Norziah, M.H. Lu, H.F.S., 2010. Effect of flaxseed oil towards physicochemical and sensory characteristic of reduced fat ice creams and its stability in ice creams upon storage. Int. Food Res. J., 17, 393-403.

Liu, J., Sempos, C.T., Donahue, R.P., Dorn, J., Trevisan, M., Grundy, S.M., 2006. Non–high-density lipoprotein and very-low-density lipoprotein cholesterol and their risk predictive values in coronary heart disease. Am. J. Cardiol., 98 (10), 1363-1368.

Lucas, E.A., Lightfoot, S.A., Hammond, L.J., Devareddy, L., Khalil, D.A., Daggy, B.P., Arjmandi, B.H., 2004. Flaxseed reduces plasma cholesterol and atherosclerotic lesion formation in ovariectomized Golden Syrian hamsters. Atherosclerosis, 173 (2), 223-229.

Marshall, R.T., Arbuckle W.S., 1996. Ice Cream.5th Ed. Chapman and Hall, New York, NY, p.23.

Marszalek, J.R., Lodish, H.F., 2005. Docosahexaenoic acid, fatty acid–interacting proteins, and neuronal function: breast milk and fish are good for you. Ann. Rev. Cell Dev. Biol., 21, 633-657.

Mathur, M.P., Roy, D.D., Dinakar, P., 2005. Textbook of Dairy Chemistry. ICAR Publication Published by DIPA, ICAR, New Delhi.

Moghadasian, M.H., 2008. Advances in dietary enrichment with n-3 fatty acids. Crit. Rev. Food Sci. Nutr., 48(5), 402-410.

Mohamed, M.S., Afifi, A.A., 2011. Effect of some polyunsaturated fatty acids rich-oils on plasma lipid profiles using normal Rats. J. Agric. Chem. Biotechnol., Mansoura Univ., (2), 49-59.

Morise, A., Sérougne, C., Gripois, D., Blouquit, M.F., Lutton, C., Hermier, D., 2004. Effects of dietary alpha linolenic acid on cholesterol metabolism in male and female hamsters of the LPN strain. J. Nutr. Biochem., 15 (1), 51-61.

Oomah, B.D., 2001. Flaxseed as a functional food source. J. Sci. Food Agric., 81 (9), 889-894.

Popa, V.M., Gruia, A., Raba, D.N., Dumbrava, D., Moldovan, C., Bordean, D., Mateescu, C., 2012. Fatty acids composition and oil characteristics of linseed (*LinumUsitatissimum L.*) from Romania. J. Agroaliment. Processes Technol., 18 (2), 136-140.

Riediger, N.D., Othman, R., Fitz, E., Pierce, G.N., Suh, M., Moghadasian, M.H., 2008. Low n-6: n-3 fatty acid ratio, with fish-or flaxseed oil, in a high fat diet improves plasma lipids and beneficially alters tissue fatty acid composition in mice. Eur. J. Nutr., 47 (3), 153-160.

Russo, G.L., 2009. Dietary n− 6 and n− 3 polyunsaturated fatty acids from biochemistry to clinical implications in cardiovascular prevention. Biochem. Pharmacol., 77 (6), 937-946.

Simopoulos, A.P., 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed. Pharmaco ther., 56 (8), 365-379.

Simopoulos, A.P., 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exper. Biol. Med., 233 (6), 674-688.

Söğüt, E., Avcı, E., Üstüner, F., Arıkan, E., 2006. The Evaluation of (TG/HDL-C) ratio as a serum atherogenic index. Türk Klinik Biyokimya Dergisi, 4 (1), 1-8.

Soltan, S.S.A.M., 2012. The effects of varieties sources of omega-3 fatty acids on diabetes in rats. Food Nutr. Sci., 3 (10), 1404.

SPSS, 1998. Statistical Package for The Social Science for Release 9.0.0, standard version SPSS. Inc.

Strolien, H.H., Kriketos, A.D., Calvert, G.D., Baur, L.A., Jenkins, A.B., 2007. Fatty acids, triglycerides and syndromes of insulin resistance. Prostaglandins and plasma lipid and lipoprotein metabolism in human: a critical review. Diabetes Care, 30 (4), 1012-1024.

Thayer, A.M., 1992. Food Additives. Chem. Eng. News, 70, 26-44.

Tou, J.C., Altman, S.N., Gigliotti, J.C., Benedito, V.A., Cordonier, E.L., 2011. Different sources of omega-3 polyunsaturated fatty acids affects apparent digestibility, tissue deposition, and tissue oxidative stability in growing female rats. Lipids Health Dis., 1 (10), 179.

Tzang, B.S., Yang, S.F., Fu, S.G., Yang, H.C., Sun, H.L., Chen, Y.C., 2009. Effects of dietary flaxseed oil on cholesterol metabolism of hamsters. Food Chem., 114 (4), 1450-1455.

USDAH, 2000. U.S Dept. Agri. Dept. Health Human Services Dietary guidelines for Americans.5th Ed. Home and Garden Bull., 232, p.112.

Vijaimohan, K., Jainu, M., Sabitha, K.E., Subramaniyam, S., Anandhan, C., Devi, C.S., 2006. Beneficial effects of alpha linolenic acid rich flaxseed oil on growth performance and hepatic cholesterol metabolism in high fat diet fed rats. Life Sci., 79 (5), 448-454.

Zommara, M.A., El-Baz, A.M., Rashed, M.A., Mansour, A.A., 2006. Health promoting effects of mixed Zabady and Bifidobacteria fermented milks fed to rats. Egypt. J. Dairy Sci. 34 (1), 47-57.

دمج زيت بذور الكتان في إنتاج مثلوج لبني وظيفي وتقييم تأثيره الصحي على الفئران

منصور عبده علي *، ابراهيم محمد محروس

[1] قسم الألبان، كلية الزراعة بالقاهرة، جامعة الازهر، مدينة نصر، القاهرة، مصر

* البريد الإليكتروني للباحث الرئيسي: DR.Mansourabdou@azhar.edu.eg

الملخص العربي

الآيس كريم من منتجات الألبان المحببة جدًا فى كل العالم. كان الهدف من هذه الدراسة ه وانتاج مثلوج لبني وظيفي غني بمحتواه من الأحماض الدهنية غير المشبعة بإستخدام تركيزات مختلفة من زيت بذور الكتان .لذلك، تم تحضير ثلاثة أنواع مختلفة من المثلوج اللبني عن طريق استبدال 25،50 و 75 ٪ من دهن اللبن (3 ٪) بزيت بذور الكتان. أظهرت نتائج توصيفات الأحماض الدهنية للمثلوج اللبني العادي والوظيفي أن أعلي تركيز للأحماض الدهنية المشبعة كان موجودًا في عينة المقارنة، حيث كان 63.72٪، بينما تم اكتشاف أقل رقم في العينة F3 (استبدال دهن اللبن بزيت بذرة الكتان بنسبة 75%) فكان 21.1٪ .في المقابل، كانت عينة المقارنة أقل قيمة للأحماض الدهنية غير المشبعة، حيث بلغت 34.96٪، في حين كان أعلي تركيز في العينة F3 حيث بلغ 77.58 ٪ .علاوةً على ذلك، زاد محتوى الأحماض الدهنية أوميغا 3 من 1.87٪ في عينة المقارنة إلي 11.97٪ لعينة F3 وأنخفضت نسبة أوميغا 6 / أوميغا 3 بشكل كبير من 2.49 لعينة المقارنة إلي 0.27 للعينة F3. هذا الإنخفاض في نسبة أوميغا 6 / أوميغا 3 كان نتيجة زيادة نسبة استبدال زيت بذور الكتان في خليط المثلوج اللبني ومع ذلك، فإن تقليل تناول أوميغا 6 وزيادة أوميغا 3 ضروري لتحسين الصحة والوقاية من الأمراض المزمنة. من أجل تقييم تأثير تغذية المثلوج اللبني الوظيفي على الدهون في مصل دم الفئران، تم تغذية المنتج لفئران التجارب ووفقاً لنتائج تحليل المصل، إنخفضت مستويات الكوليسترول الكلي، الدهون الثلاثية، LDL، VLDL وقلت مستويات الخطورة 1 و 2 و LDL / HDL مقارنة بعينة المقارنة (CII).

الكلمات المفتاحية: مثلوج لبني وظيفي، أوميغا 3، الأحماض الدهنية غير المشبعة.