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

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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 omega-3 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-conscious 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 decreasing consumption of total and saturated fat and cholesterol by substituting butterfat with vegetable oils such 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 its 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


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:

\[
\text{Cholesterol (mg/dL)} = \frac{A_{\text{of the tested sample}}}{A_{\text{of the standard solution}}} \times 206
\]

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:

\[
\text{Concentration of HDL-cholesterol (mg/dL) in supernatant} = A_{\text{sample}} \times 325 \quad \text{Where: A= Absorbance at 546 nm.}
\]

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

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

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

**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:

\[
\text{VLDL-cholesterol (mg/dL)} = \frac{\text{Triglycerides}}{5}
\]

**Determination of Triglycerides**

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

\[
\text{Triglycerides (mg/dL)} = \frac{A_{\text{of the tested sample}}}{A_{\text{of the standard solution}}} \times 202
\]

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
- Food efficiency ratio = \( \frac{\text{Growth rate, g day}}{\text{Food intake, g day}} \)
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 $\omega-3$ 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.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Common name</th>
<th>% Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C16:0)</td>
<td>Palmitic acid</td>
<td>5.0</td>
</tr>
<tr>
<td>(C18:0)</td>
<td>Stearic acid</td>
<td>3.9</td>
</tr>
<tr>
<td>(C18:1) $\omega$9</td>
<td>Oleic acid</td>
<td>18.91</td>
</tr>
<tr>
<td>(C18:2) $\omega$6</td>
<td>Linoleic acid</td>
<td>14.83</td>
</tr>
<tr>
<td>(C18:3) $\omega$3</td>
<td>$\alpha$-Linolenic acid</td>
<td>55.93</td>
</tr>
<tr>
<td>(C18:1) $\omega$7</td>
<td>Vaccenic acid</td>
<td>1.0</td>
</tr>
<tr>
<td>C18:4 $\omega$3</td>
<td>Steardionic acid</td>
<td>0.14</td>
</tr>
<tr>
<td>C20:0</td>
<td>Arachidic acid</td>
<td>0.12</td>
</tr>
<tr>
<td>C20:1</td>
<td>Gadoleic acid</td>
<td>0.1</td>
</tr>
<tr>
<td>Non Identified Fatty acids</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Total Saturated Fatty Acids (TSFA)</td>
<td>9.02</td>
<td></td>
</tr>
<tr>
<td>Total Unsaturated Fatty Acids (TUSFA)</td>
<td>90.91</td>
<td></td>
</tr>
<tr>
<td>Omega6/Omega3 Ratio</td>
<td>0.26</td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Skim Milk</td>
<td>54.74</td>
<td>55.86</td>
<td>57.00</td>
<td>58.12</td>
</tr>
<tr>
<td>Skim Milk Powder</td>
<td>7.20</td>
<td>7.20</td>
<td>7.20</td>
<td>7.20</td>
</tr>
<tr>
<td>Cream40%</td>
<td>7.50</td>
<td>5.63</td>
<td>3.75</td>
<td>1.87</td>
</tr>
<tr>
<td>Flaxseed Oil</td>
<td>0.00</td>
<td>0.75</td>
<td>1.50</td>
<td>2.25</td>
</tr>
<tr>
<td>Sugar</td>
<td>15.00</td>
<td>15.00</td>
<td>15.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Fruit’s pulp*</td>
<td>15.00</td>
<td>15.00</td>
<td>15.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Stabilizers</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Emulsifiers</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Vanillin Essence</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
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.

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

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 %CMC, 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](image.png)

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-3 fatty 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, the incorporation 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.

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

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.

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

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.

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

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

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.

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

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

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).
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 indices 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.

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*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.


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

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الملخص

المثلوج من منتجات الـلبن诲بة جدًا في كل العالم، كان الهدف من هذه الدراسة هو إنتاج مثلوج لبنى وظيفي غني بمحتواه من الـحمض الدهني غير المشبعة باستخدام 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 (%D) بزيت بذور الكتان (%C). أظهرت نتائج توصيفات الـحمض الدهني للمثلوج اللبني العادي والوظيفي أن أعلى تركيز للـحمض الدهني المشبعة كان موجودًا في عينة المقارنة، حيث كان 63.72% بينما تم اكتشاف أقل رقم في العينة F3 (استبدال 75% من دهن اللبن بزيت بذرة الكتان) بنسبة 21.1%.

كانت عينة المقارنة أقل قيمة للأحماض الدهنية غير المشبعة، حيث بلغت 34.96% ، في حين كان أعلى تركيز في العينة F3 حيث بلغ 77.58%.

بالإضافة إلى ذلك، زاد محتوى الـحمض الـأوميغا 3 من 1.87% في عينة المقارنة إلى 11.97% في عينة المقارنة إلى 0.27% للعينة F3. هذا الإختلاف في نسبة أوميغا 6 أوميغا 3 كان نتيجة زيادة نسبة استبدال زيت بذور الكتان في الخليط المثلوج اللبني.

لذلك، فإن تقليل نسباً أوميغا 6 ويزيادة أوميغا 3 ضروري لتحسين الصحة الوقائية من الأمراض المزمنة. من أجل تقييم تأثير تغذية المثلوج البنني الوظيفي على الدهون في مصل دم الفئران، تم تغذية المثلوج النباتي وفقًا لنتائج تحليل المصل، وتخلصت مستويات الكوليسترول الكلي، الدهون الثلاثية، VLDL، LDL، LDL / HDL مقارنة عينة المقارنة (CII).

الكليات المفتاحية: مثلوج لبنى وظيفي، أوميغا 3، الأحماض الدهنية غير المشبعة.