Effect of Raw and Heated Flaxseed (Linum Usitatissimum L.) On Blood Lipid Profiles in Rats

Saman Khalesi[a], Rosita Jamaluddin[a*], Amin Ismail[ab]

[a] Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia. SK: Undergoing MSs in Nutritional Sciences; RJ: PhD in Nutrition, Senior Lecturer.

[b]Laboratory of Halal Science Research, Halal Products Research Institute, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. AI: PhD in nutritional sciences, Professor.

Abstract

Flaxseed is a nutrient rich seed and lipid profiles improving effect of it has long been studied. Effect of heating as a part of food processing on its beneficial characteristics is not clarified in literature. This study aims to provide complementary information on effect of different dosages of raw and heated flaxseed on lipid profiles. Sprague Dawley rats were fed with 10%, 20% or 30% of either raw or heated flaxseed in the basal diet for 30 days. Total cholesterol significantly reduced in all flaxseed groups and high density lipoprotein cholesterol significantly increased in 20% raw and 30% raw and heated flaxseed groups. Significant reduction of low density lipoprotein cholesterol only observed in 30% raw flaxseed groups. It is concluded that 30 days consumption of flaxseed may significantly reduce total cholesterol and increase high density lipoprotein cholesterol in blood. Oven heating may not have significant effect on lipid profile improving effect of flaxseed.

Key words: Blood lipid profiles, Flaxseed, Heating,

1. Introduction

After the industrial revolution, the human diet has changed from unrefined whole grains and vegetables with high fiber to refined grains with low fiber content and more animal products. These changes beside the reduced physical activity of human due to mechanization caused an increase in the risk of some chronic diseases related to diet and lifestyle like obesity, diabetes, cardiovascular and heart related diseases. During the past decades many studies have focused on improving lipid profiles (as one of the most important risk factors of chronic diseases) by planning a better diet or introducing herbal treatments. Thus, many plants were proposed to have health benefits in reducing blood lipid profile. Flaxseed, of *Linum usitatissimum L.*, is a member of the *Linaceae* family with seed's color ranging from light to reddish brown (1). Usage of flaxseed is dated around 8000 B.C.E., in the Fertile Crescent (Syria, Turkey and Iran)(2). Flaxseed contains about 42.16 g fat (approximately 57% of total fat is α-linolenic acid, ALA, 18:3 ω-3) and 27.3 g fiber per 100 g (3). Flaxseed is also a rich source of lignans which are one of the major groups of phytoestrogens that have antitumorigenic and antioxidant properties (4). Lipid profile improving effect of flaxseed has long been studied and many literatures have related its effect to high fiber, ALA and lignans content of flaxseed (5, 12-15). Many studies reported that flaxseed may have protective effect against diseases like CVD (5-7), reducing the risk of cancers (8-10), reducing the incident and progress of diabetes (11) and many other health benefits.

Flaxseed can be consumed in either raw or cooked form in daily diet. However, the effect might not be the same after heating flaxseed due to chemical and physical changes that may occur in seed with regard to the oxidation or thermal deformation of ALA, vitamins and other components. Besides, the different dosages of flaxseed may have different characteristics in the lipid lowering effect. There is not enough literature on the effect of heating (as a step of food preparation and processing) on the lipid improving effect of flaxseed and also no strong comparison on the effect of different dosages of flaxseed consumption. Moreover, numerous studies used flaxseed in muffins and bread (mostly baked at 160-190°C) without considering the possible effect of heating on flaxseed characteristics. Thus this study aims to clarify the effect of heated and raw flaxseed in concrete dosages on blood lipid profiles in rats to provide a comprehensive comparison.

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2. Methods

2.1. Flaxseed source

Flaxseed used in this experiment was the small brown genus *linum* flax and was purchased in 500 g packages imported from New Zealand by Radiant Code Sdn. Bhd., Malaysia. All the packages were kept at 4℃ until usage.

2.2. Animals

Male Sprague Dawley rats, 7-8 weeks of age and 170 to 180 g of weight were kept at room temperature with 12 hours light-dark cycle in the animal research house. All rats were housed in plastic cages with wood chip bedding. The study protocol was approved by Animal Care and Use Committee (ACUC) of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

2.3. Diet preparation

The standard or basal commercial diet for rats purchased from Gold Coin Feed Mills (M) Sdn. Bhd., Malaysia, and was finely ground using commercial grinder (A&D, Japan). Raw flaxseed was separated into two batches; first one was finely ground with a commercial grinder and the other batch was heated for 15 min using electric oven at 170℃ before grinding. Each of the two batches was added with a concrete amount of ground basal diet after grinding to make up 10%, 20% and 30% flaxseed in the diet. Water was added to the mixture to make pellets and dried in laboratory oven at 40℃ for overnight (in animal house). Food preparation was daily and the prepared food was kept in the refrigerator below 20℃ to prevent spoilage.

2.4. Experimental study

Rats were divided into 7 groups and were first fed with basal diet (BS) of commercial mouse pellet (Golden Coins mouse pellets), for 7 days to adjust to the environment (acclimatization period). Rats were fed with the experimental diet for a period of 30 days. Flaxseed diet was prepared by adding 10%, 20% and 30% of flaxseed (FL) to BS as following:

Control: BS (control diet); H10: (10% heated FL in BS); H20: (20% heated FL in BS); H30: (30% heated FL in BS); R10: (10% raw FL in BS); R20: (20% raw FL in BS); R30: (30% raw FL in BS). **Table 1** illustrates the approximate nutritional components of different groups. Food was presented more than the calculated amount and at the end of each day. The remnant was measured and deducted from the food presented to calculate the amount of food consumed. Water was given ad libitum throughout the study

2.5. Blood analysis

Blood samples were collected at baseline (day 0 of treatment) and after 30 days of treatment. Food was removed for 12 hours and rats where anesthetized using diethyl ether and blood was collected in vacutainers containing Ethylenediaminetetraacetic acid (EDTA). At the end of blood withdrawal rats were sacrificed using diethyl ether anesthesia.

Blood plasma was separated using centrifuge at 4℃ for 10 min at 3000 rpm and was collected in Hitachi cups and stored at -80℃ until analyzed. Plasma lipid profiles were analyzed enzymatically using Hitachi chemical analyzer (Roche Diagnostic, Japan), before and after the experiment.

2.6. Statistical analysis

Data was expressed by mean ± SD. Data analysis was performed using Minitab software version 15.1.1.0 (Minitab Inc. USA). Before and after treatment differences were measured using paired t-test and the probability of a Type error was set at 5%. Between groups differences were analyzed by one-way analysis of variance (ANOVA), followed by one-way multiple comparison of Tukey and *p* values lower than 0.05 were considered as statistically significant differences.

3. Results

3.1. Food intake and weight changes

Overall each rat consumed 25.67 ± 1.67 g food in this study. As shown in **Table 2**, although food consumption was slightly lower in groups with higher percentage of flaxseed (maybe due to the fact that flaxseed has high level of fat and fiber), no significant difference was observed between the average food intake between any treatment group and control group ($p > 0.05$). Also within groups analysis of food intake showed no significant difference.

Rat's weight positively increased during the treatment period in all groups with the average percentage of 52.61 ± 15.10. No significant difference in the percentage of weight gain between and within groups was observed as mentioned in Table 2 ($p > 0.05$).

3.2. Blood lipid profiles analysis

Analysis of blood lipid profiles before treatment showed no significant differences among all groups in all parameters ($p > 0.05$). The comparison of blood lipid profiles of control group after treatment period revealed a slight increase in total cholesterol (TC), triacylglycerol (TG) and low density lipoprotein cholesterol (LDL-C) and a mild decrease in high density lipoprotein cholesterol (HDL-C) but these changes were not significant (**Table 3**). A significant reduction ($p < 0.05$) of total cholesterol was observed after treatment period for all flaxseed diet groups with 30% raw FL group having the highest percentage of change (24.8% reduction) followed by 30% heated FL group (22.0% reduction) and 20% raw FL group (21.7% reduction; Table 3). Heated 20% FL group and heated and raw 30% FL group showed significantly lower blood total cholesterol compared to control group but no significant difference was observed between any flaxseed groups on total cholesterol (Table 3). A significant increase ($p < 0.05$) in blood HDL-C was observed in rats after treatment with 20% raw FL group (3.6 % increases). In 30% FL group both raw and heated flaxseed groups also showed a significant increase in HDL-C (4.81% increases and 6.0% increases respectively, Table 3). The 30% FL group either heated or raw showed significantly higher blood HDL-C compared to control group. No significant difference was observed between any flaxseed groups on HDL-C concentration (Table 3).

Except for raw 30% FL group, LDL-C did not significantly changed after treatment period. In 30% raw FL group, LDL-C decreased 7.6 % ($p < 0.05$). After treatment analysis also showed no significant difference between LDL-C concentrations of control with any treatment group (Table 3). Blood triacylglyceride did not significantly change (although slightly decreased) in all flaxseed diet group after treatment period ($p > 0.05$). No effect of heating was observed on blood TG level after treatment (Table 3). LDL-C: HDL-C ratio also slightly decreased after treatment period in flaxseed groups. This reduction was significant in 30% raw FL group ($p < 0.05$). However, the differences between before and after treatment LDL-C: HDL-C ratio was not significant in other groups ($p > 0.05$). TC: HDL-C ratio on the other hand, significantly reduced in all flaxseed groups after treatment ($p < 0.05$) with 30% raw FL group having the lower ratio compared to other groups (Table 3).

4. Discussion

Overall consumption of flaxseed significantly reduced total cholesterol which is similar to the previous findings (5, 16) It also significantly increased HDL-C concentration similar to the report by Daleprane et al. (15). The significant reduction of TC and TC: HDL-C ratio was observed in all treatment groups however; HDL-C only increased significantly in 20% raw and 30% raw and heated FL groups. By increasing the dosage of flaxseed in the diet the blood improving effect of flaxseed became more significant. The reason why 20% raw FL group showed significant HDL-C increasing effect but the 20% heated FL group did not show the effect, is not clear although it can be assumed that heating may have slight component degradation effect. The significant reduction of LDL-C which was only observed in the 30% raw FL group may also be due to the same effect mentioned however; no significant difference of blood TC lowering and HDL-C increasing effect of flaxseed were observed between raw and heated groups which can be concluded that heating (at 170°C for 15 min) does not have significant effect on flaxseed blood lipid profile improving effect.

Flaxseed has approximately 57% ALA in 100g of oil (3, 17) and is one of the richest sources of ALA. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are converted from ALA in the body, have anti-inflammatory factor and have effect on reducing the risk of many chronic diseases like atherosclerosis, cardiovascular disease (CVD), cancer and hyperlipidemia (18,19). Although in literature the conversion efficacy of ALA to EPA and DHA is mentioned to be below 0.6% in animal models (20), many literatures have related the hypocholesterolemic effect of flaxseed to high ALA content of flaxseed oil (13, 21, 22). Flaxseed is also the richest source of Secoisolariciresinol diglucoside (SDG), approximately 1% of flaxseed dry weight is SDG (23). Literatures also suggest that flaxseed lignans have blood lipid profiles improving effects (7, 24). Lignans are estrogen like components which also have antioxidant activity in reducing the oxidative damage in many diseases like heart disease, cancer and diabetes (25-27). Besides, the high fiber content of flaxseed (approximately 27 g fiber per 100 g) can also contribute to the hypocholesterolemic effect of flaxseed. Two mechanisms for the effect of dietary fiber are proposed. First that dietary fiber can reduce oxidation of glucose and lipids, besides it can provide a healthy intestinal environment.

Another mechanism is that dietary fiber can reduce inflammation by changing adipocytokines in adipose tissue and reducing blood cholesterol by interrupting enterohepatic circulation of cholesterol or by fat binding properties (28). Many studies unanimously addressed different sources of fiber as cholesterol lowering and lipid profile improving factor (6, 29). Therefore the lipid profile improving effect of flaxseed can be mostly due to the existence of high level of ALA, lignans and fiber in flaxseed. Thus by increasing the dosage of flaxseed in diet the blood lipid profile improving effect of flaxseed increases.

This study concluded that ground flaxseed intervention for a period of 30 days may have lipid profile improving effect especially if dosages between 20 and 30% of FL in the diet consumed. Moreover, heating at 170℃ for 15 min does not have significant effect on flaxseed lipid lowering characteristics. Further studies need to be done on the effect of higher time and temperature of heating (if applicable due to the burning and browning of flaxseed in higher degrees) and different methods of preparation of foods with flaxseed.

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Table 1. Nutritional components of diet

Properties	**Level (g/100g food)**				
	Control group	**Pure FL**	**10% FL**	**20% FL**	**30% FL**
Protein	22	20	21.8	21.6	21.6
Total fat	4	39.5	7.5	11.3	11.3
Total carbohydrate	51.5	31.5	49.5	47.5	47.5
Crude fiber	5.5	25	7.45	9.4	9.4
α-linolenic acid	-	22.81	2.28	4.56	4.56
Lignans	-	1.0	1.0	2.0	2.0
Energy	330	561.5	353.1	378.1	378.1

Basal diet and pure flaxseed ingredients are approximate, base on information provided by the distributer company (Source: Gold Coin Feed Mills (M) Sdn. Bhd. and Radiant Code Sdn. Bhd. Malaysia)

Table 2. Effect of flaxseed diet on food and energy intake and weight change (mean ± SD) [1]

Groups	**Food intake (g/rat/d)**	**Energy intake (Kcal/rat/d)**	**Weight change (%)**
C	27.09±1.30[a]	89.39±4.28[b]	54.11±19.42[c]
H10	26.09±2.45[a]	92.09±17.25[b]	60.04±9.94[c]
H20	25.28±1.43[a]	95.45±10.77[b]	62.85±12.17[c]
H30	25.38±1.44[a]	101.26±5.74[b]	62.40±12.90[c]
R10	25.81±1.74[a]	91.07±6.13[b]	54.11±19.42[c]
R20	25.09±2.50[a]	94.68±9.4[b]	56.01±19.62[c]
R30	24.94±0.88[a]	99.50±3.51[b]	48.66±8.58[c]

[1]Values in same column with similar superscript letters are not significantly different (One way ANOVA; $p > 0.05$). C (control); H10 (heated 10% FL); H20 (heated 20% FL); H30 (heated 30% FL); R10 (raw 10% FL); R20 (raw 20% FL); R30 (raw 30% FL).

Table 3. Lipid profile changes before and after treatment (mean±SD)

Groups		Blood lipid profiles (mmol/L)						
		Before treatment	After treatment	% changes		Before treatment	After treatment	% changes
R10					**H10**			
	TC	**1.53 ± 0.18**	**1.34 ± 0.24*** ^{ab}	**-12.41**	**TC**	**1.56 ± 0.28**	**1.37 ± 0.26*** ^{ab}	**-12.17**
	TG	**0.40 ± 0.07**	**0.38 ± 0.06**	**-5.00**	**TG**	**0.39 ± 0.05**	**0.38 ± 0.06**	**-2.63**
	HDL-C	**0.85 ± 0.06**	**0.84 ± 0.04**	**-1.17**	**HDL-C**	**0.80 ± 0.05**	**0.82 ± 0.04**	**2.50**
	LDL-C	**0.26 ± 0.02**	**0.26 ± 0.02** ^{cd}	**0.00**	**LDL-C**	**0.26 ± 0.03**	**0.26 ± 0.02** ^{cd}	**0.00**
	TC:HDL-C	**1.84 ± 0.18**	**1.55 ± 0.25***	**-0.15**	**TC:HDL-C**	**1.95 ± 0.29**	**1.72 ± 0.27**	**-0.11**
	LDL-C:HDL-C	**0.31 ± 0.02**	**0.31 ± 0.03** ^{fg}	**0.00**	**LDL-C:HDL-C**	**0.34 ± 0.02**	**0.32 ± 0.03** ^{ef}	**-0.05**
R20					**H20**			
	TC	**1.61 ± 0.18**	**1.26 ± 0.12*** ^{b}	**-21.73**	**TC**	**1.63 ± 0.18**	**1.38 ± 0.12*** ^{ab}	**-15.33**
	TG	**0.40 ± 0.02**	**0.38 ± 0.05**	**-5.00**	**TG**	**0.40 ± 0.07**	**0.37 ± 0.07**	**-7.50**
	HDL-C	**0.82 ± 0.05**	**0.85 ± 0.04***	**3.65**	**HDL-C**	**0.80 ± 0.09**	**0.84 ± 0.02**	**5.00**
	LDL-C	**0.27 ± 0.02**	**0.26 ± 0.01** ^{cd}	**-3.70**	**LDL-C**	**0.27 ± 0.01**	**0.27 ± 0.02** ^{cd}	**0.00**
	TC:HDL-C	**2.00 ± 0.20**	**1.49 ± 0.14***	**-0.25**	**TC:HDL-C**	**2.04 ± 0.31**	**1.65 ± 0.14***	**-0.19**
	LDL-C:HDL-C	**0.33 ± 0.02**	**0.31 ± 0.02** ^{fg}	**-0.06**	**LDL-C:HDL-C**	**0.34 ± 0.04**	**0.32 ± 0.03** ^{efg}	**-0.05**
R30					**H30**			
	TC	**1.61 ± 0.09**	**1.21 ± 0.25*** ^{b}	**-24.84**	**TC**	**1.63 ± 0.19**	**1.27 ± 0.16*** ^{b}	**-22.08**
	TG	**0.40 ± 0.03**	**0.37 ± 0.05**	**-7.50**	**TG**	**0.39 ± 0.05**	**0.36 ± 0.03**	**-7.69**
	HDL-C	**0.83 ± 0.06**	**0.87 ± 0.06***	**4.81**	**HDL-C**	**0.83 ± 0.02**	**0.88 ± 0.05***	**6.02**
	LDL-C	**0.26 ± 0.01**	**0.24 ± 0.02*** ^{d}	**-7.69**	**LDL-C**	**0.25 ± 0.02**	**0.24 ± 0.02** ^{d}	**-4.00**
	TC:HDL-C	**1.97 ± 0.18**	**1.41 ± 0.23***	**-0.28**	**TC:HDL-C**	**1.93 ± 0.20**	**1.43 ± 0.18***	**-0.25**
	LDL-C:HDL-C	**0.32 ± 0.02**	**0.28 ± 0.04*** ^{g}	**-0.12**	**LDL-C:HDL-C**	**0.31 ± 0.02**	**0.29 ± 0.03** ^{fg}	**-0.06**
C								
	TC	**1.56 ± 0.18**	**1.57 ± 0.17** ^{a}	**0.64**				
	TG	**0.38 ± 0.06**	**0.40 ± 0.07**	**5.26**				
	HDL-C	**0.81 ± 0.05**	**0.80 ± 0.03**	**-1.23**				
	LDL-C	**0.24 ± 0.02**	**0.24 ± 0.03** ^{c}	**0.00**				
	TC:HDL-C	**1.87 ± 0.13**	**1.94 ± 0.26**	**0.05**				
	LDL-C:HDL-C	**0.30 ± 0.03**	**0.31 ± 0.03** ^{e}	**0.03**				

*Significant difference between before and after treatment performing paired t test ($p < 0.05$). Between groups values with different superscript letters are significantly different ($p < 0.05$). C (control); H10 (heated 10% FL); H20 (heated 20% FL); H30 (heated 30% FL); R10 (raw 10% FL); R20 (raw 20% FL); R30 (raw 30% FL).