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Influence of Functional Sweet White Lupin Biscuits on Lipid Profile and Food Efficiency of Induced Hyperlipidemia Rats

Rasha Mousa Ahmed Mousa[1]

[1] Home Economic Department, Faculty of Specific Education, Assiut University, Assiut, Egypt

Correspondence: Rasha Mousa Ahmed Mousa, Home Economic Department, Faculty of Specific Education, Assiut University, Assiut, Egypt. E-mail: rashamousa@aun.edu.eg; rshmousa73@gmail.com

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Abstract

In recent years, functional foods have attracted much interest to prevent nutrition-related diseases such as hyperlipidemia and weight gain. In this regard, this study was designed to examine the effect of use sweet white lupin (SWL) oil and flour with/without germination as a source of active healthy components to prepare functional biscuits for lowering blood lipids and growth. Functional biscuits were formulated by replacing wheat flour and butter in biscuit formulae by SWL extracted flour and SWL oil in the range of 20-30% (w/w) and 30-40% (v/w), respectively. Results indicated that the feed of hyperlipidemic rats on diets supplemented with different functional SWL biscuits for 6 weeks significantly ($P < 0.05$) reduced serum total cholesterol, triglycerides, low density lipoproteins, very low density lipoproteins, ratio of total cholesterol/high density lipoproteins cholesterol, ratio of low density lipoproteins/high density lipoproteins cholesterol and atherogenic index. Furthermore, the feed of functional SWL biscuits significantly reduced the body weight gain of rats and their food efficiency compared to that of rats fed on hyperlipidemic diet. On the other hand, there was an increase in the value of high density lipoproteins cholesterol and its ratio with total cholesterol. All these findings supported that the addition of 25% germinated SWL flour and 35% or 40% germinated SWL oil in biscuits gave interested results compared to the common wheat biscuits. Therefore, the proposed functional SWL biscuits could be able to regulate the blood cholesterol and the body growth levels of individuals and patients.

Keywords: biscuits, cholesterol, food efficiency, germination, lipoprotein, lupins, triglycerides

1. Introduction

Hyperlipidemia is a general term for the high concentrations of any or all types of the lipids in serum (lipid profile) that include cholesterol low density lipoproteins (LDL-cholesterol; LDL-c), cholesterol very low density lipoproteins (VLDL-cholesterol; VLDL-c), cholesterol high density lipoproteins (HDL-cholesterol; HDL-c), total cholesterol (TC) and triglycerides (TG) (National Cholesterol Education Program (NCEP), 2002). Cholesterol and triglycerides levels are measured in milligrams (mg) of cholesterol per deciliter (dL) of blood in the United States and some other countries while Canada and most European countries measure cholesterol in millimoles (mmol) per liter (L) of blood (Integrated Guidelines, 2011); Consider these general guidelines, the recommended normal ranges of LDL-c, HDL-c, TG and TC are < 100 mg/dL (2.5 mmoL/L), > 45 mg/dL (1.6 mmol/L), < 150 mg/dL (1.7 mmol/L) and < 200 mg/dL (5.1 mmol/L), respectively. The ratios of TC/HDL-c, LDL-c/HDL-c (risk ratio; RR), 100.HDL-c/TC (HTR%) and atherogenic index (AI) are predictors of coronary risk (National cholesterol education program, 1994). Meanwhile, the changes in eating habits including high fat and low fiber diets increased also the risk of obesity and overweight. Risk of these diseases increases steadily with increasing the values of body mass index (BMI) or food efficiency ratio (FER).

Legumes played an important role for improving the lipid profile in humans since they are rich sources of protein, fiber, certain minerals and vitamins (ELMaki et al., 2007). Among the legumes, soybean and its protein fractions appeared a significant reduction in TC and LDL-c through the modulation of genes related to the lipid metabolism (Anderson, Johnstone, & Cook-Newell, 1995). However, the presence of isoflavones that have potentially harmful effects on humans (Sirtori, 2001) reduced the impact of soybeans. Other seeds such as peas, lentils, chickpeas, beans and lupin are also investigated due to their chemical composition and great potential in the prevention of lipid disorders (Duranti, 2006; Sirtori et al., 2004; Smith et al., 2006). Lupin is the only another protein-rich grain legume and contains low levels of isoflavons. More than 300 lupin species were described, but

only five species are cultivated. One of the most important species is the sweet white lupin (SWL); It is most commonly found in Mediterranean countries especially in Egypt, Portugal, Greece, and Italy (Hassanein, El-Shami, & El-Mallah, 2011).

The chemical composition of SWL confirmed that it is a good source of nutrients, not only proteins but also dietary fibers, minerals, vitamins and healthy oils. The amount of SWL oil was found to be in the range of 5-20% (v/w) of total lupin grain. SWL Oil was characterized by the low percent of saturated fatty acids (≤ 10%), absence of trans fatty acids, absence of cholesterol and high percent of healthy unsaturated fatty acids (≥ 90%) including 30-50% oleic acid, 15-45% linoleic acid and 3-11% linolenic acid (Gravelle, Barbut, & Marangoni, 2012; Stortz, Zetzl, Barbut, Cattaruzza, & Marangoni, 2012; Tarancón, Fiszman, Salvador, & Tárrega, 2013). Lupin flour has always been a rich of proteins and dietary fibers with the range of 36-52% and 30-40% (w/w), respectively (Mohamed & Rayas-Duarte, 1995). The SWL protein has two major fractions, albumins and globulins in a ratio of 1:9, respectively. In turn, the globulins consisted of the two classical major storage proteins called conglutins β and two minor distinct protein types, conglutins γ and ξ (Blagrove & Gillespie, 1975). Conglutin γ is an oligomeric lupin seed glycoprotein comprising 5% of the total lupin proteins (Duranti, Restani, Poniatowska, & Cerletti, 1981). However, the presence of anti-nutrients reduced the broad impact of lupin grains for improvement of the public health.

Germination of legumes is a simple, low-cost and effective process for decreasing the levels of anti-nutrients and for achieving desirable changes in nutritional and sensory characteristics through a variety of reactions including synthesis, degradation and transformation of biomolecules during transformation of seeds into plants. To improve the quality of SWL seeds, germination was used to change protein, lipid and carbohydrate levels (Dueñas, Hernández, Estrella, & Fernández, 2009). Analysis of the oil extract after germination revealed that the concentrations of phytosterols and total polyphenols were increased (Rumiyati & James, 2013). In addition, the concentration of anti-nutritional factors such as phytate, trypsin inhibition, α-galactoside and alkaloids in lupin were significantly diminished by germination (Cuadra et al., 1994). However, no study was undertaken to demonstrate the anti-hyperlipidemic activity of the germinated sweet white lupin (Lupinus albus; SWL) formulated in food products such as biscuits. Biscuits are the most common food product consumed by the upper, middle and lower income countries (Monteiro, Moubarac, Cannon, Ng, & Popkin, 2013).

According to most definitions, “functional foods” are foods that provide a health benefit beyond basic nutrition (Hassan, Rasmy, Foda, & Bahgaat, 2012). Fortified foods, along with other enhanced or enriched foods, are considered functional foods. The individual and/or simultaneous substituting with SWL extracted flour and SWL oil to prepare functional SWL biscuits was previously studied in the range of 20-30% (w/w) and 30-40% (v/w), respectively by Mousa (2014). It was found that the simultaneous substitution with germinated SWL flour and oil raised significantly the values of protein, fiber and total phenolic compounds (TPCs). However, the proposed substitution showed marked reductions in the values of fat, carbohydrate, calorie and Na/K ratio. The sensory acceptability of functional SWL biscuits showed acceptable attributes of taste, appearance, texture and aroma with an observable improvement in the color of biscuits. Therefore, the present investigation was designed to evaluate the hypolipidemic activity (Youssef, Youssef, & Mousa, 2014) and food efficiency of functional SWL biscuits on hyperlipidemia albino rats. Functional SWL biscuits were prepared by simultaneous substitution of butter and wheat flour with sweet white lupin (SWL) oil and extracted flour with/without germination.

2. Materials and Methods

2.1 Materials

Sweet white lupin grains (Lupinus albus, Termis), refined wheat flour (Triticum Aestivum, 72% extraction hard red winter), butter (Cow's milk ≥ 80% fat), sugar powder (100% pure cane sugar), sodium bicarbonate, ammonium bicarbonate, skim milk powder (Defatted Cow's milk powder), salt mixture, orange (*Citrus sinensis* (L.)) peel powder, corn starch, corn oil and animal fats were procured from local market. Methanol, n-hexane, ether, nitric acid and perchloric acid were obtained from Fluka company Co. (Germany). Casein, 85% pure cholesterol, vitamin mixture, choline chloride, cellulose and cholic acid were obtained from Sigma Chemical Co (Germany). Kits of total cholesterol, HDL-cholesterol, and triglycerides were obtained from Stanbio Laboratory, Texas, USA. Double distilled water was used for the chemical analysis.

2.2 Germination of Sweet White Lupin (SWL) Grains

Prior to germination, clean lupin grains (300.0 g) were soaked in 2 L of distilled water at room temperature in the dark for 18 h. Following this the soaking water was decanted and the grains were twice rinsed with water. Then, grains were spread on dishes lined and covered with moistened paper towels. These dishes were placed on trays in an incubator set at 25 ºC and relative humidity (RH) 90-95%. The seeds in dishes were germinated for 3

days (Mousa, 2014). During the germination period, grains were washed twice per day with distilled water. At the end, the grains were dried under the sun. The above process was done in three replications to confirm the precision of the results. Finally, the germinated SWL grains or ungerminated SWL grains were ground and then sieved thrice through 10 mesh to flour. Grinding was done in Ushamixer Grinder. The obtained flours were stored in the dark at 4 °C prior to use.

2.3 Simultaneous Procuration of SWL Oil and Extracted Flour

A portion of the obtained germinated/ungerminated SWL flour (200.0 g) was exposed for oil extraction. The oil was extracted by n-hexane using a Buchi E-816 Soxhlet extraction unit (Switzerland). The weighed samples were placed into a thimble and put on pre-weighed extraction cups that placed on the extraction chamber in the soxhlet (Rumiyati & James, 2013). Consequently, the oil was extracted by n-hexane for 60 min. The extracted oil samples were kept in well stoppered dark containers under 4 °C (to prevent their autoxidation) until use as a fat replacer in the formulated biscuits. Simultaneously, the remained flour amount from oil extraction was dried over anhydrous sodium sulphate and the solvent was removed off by rotatory evaporator under vacuum. The dried extracted flour was cooled, weighed and used for the partial substitution of wheat flour in the formulated biscuits.

2.4 Preparation of Functional SWL Biscuits

The functional SWL biscuits were prepared as cited in Table 1 and were previously described by Mousa (2014). For the preparation of control/reference wheat biscuit (F1), butter (10.0 g) and sugar powder (20.0 g) were creamed together for 3 min in a mixer at 60 rpm. Then, 2.0 g of skim milk powder, 1.0 g of salt, 0.4 g of sodium bicarbonate, 1.5 g of ammonium carbonate and 5.0 g of orange peel powder were added in water (20.0 mL) and mixed together for 8 min at 125 rpm. The wheat flour (100.0 g) was added to the above mixture and mixed again for 3 min at 60 rpm. The dough was sheeted to a 3.5-mm thickness and cut with a biscuit cutter (50-mm diameter). The biscuits were baked on an aluminium tray in an electric oven at 200 ºC for 5 min. They were cooled for 30 min at room temperature and stored in low density polyethylene bags until further use.

For the preparation of functional wheat-SWL biscuits, six formulations (F2-F7) were prepared by the replacement of wheat flour with SWL in the presence of other ingredients (e.g. skim milk powder, orange peel powder, etc.) as described above. In these formulations, F2 and F3 samples were prepared by the simultaneous variation of wheat flour and butter with 20% SWL extracted flour and 30% SWL oil without germination and after germination, respectively. After that, the other remained samples (F4-F7) were formulated by the simultaneous variation with germinated SWL extracted flour in the range of 20-30% (w/w) and germinated SWL oil in the range of 30-40% (v/w).

Table 1. Variations in the component of formulated biscuits

Sample*	**Wheat Flour (g)**	**SWL Flour (g)**	**Butter (g)**	**SWL oil (mL)**	**Water (mL)**
F1 (control biscuit)	100.0	---	10.0	---	20.0
F2 (without germination)	80.0	20.0	7.0	3.0	24.0
F3 (with germination)	80.0	20.0	7.0	3.0	24.0
F4	75.0	25.0	7.0	3.0	26.0
F5	70.0	30.0	7.0	3.0	27.0
F6	75.0	25.0	6.5	3.5	26.0
F7	75.0	25.0	6.0	4.0	26.0

* Other ingredients of the formulations (F2-F6) are the same as described in the text for F1.

2.5 Rats Experimental Design

Ninety adult male white albino rats (Sprague) weighing between 100 and 120 grams were obtained from the animal house of King Fahad Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. The animals were housed as groups in wire cages under standard laboratory conditions (20-25 ºC, 40-60% humidity,

10-12 hours light/dark cycle). The animals were given free access to diet and water during the feeding period. Diet and water were given *ad- libitum* for six weeks. Before starting the experiment, the rats were fed for a week as adaptation period for acclimatization. Body weight and food intake were daily weighed through the experimental feeding period.

The rats were randomly divided into 9 groups of 10 rats each. Each rat was ranked on the tail to differentiate between animals. Daily administration was continued for 6 weeks. Group (1) involved 10 rats that were fed by the basal diet only (a negative control). The basal diet used is outlined in Tables 2 and 3 (Youssef et al., 2014). Group (2) received hyperlipidemic diet (Table 4) and served as a positive control. Group (3) received hyperlipidemic diet contained 10% (w/w) wheat flour biscuits (sample F1). Group (4) received hyperlipidemic diet contained 10% (w/w) of wheat-ungerminated SWL biscuits (sample F2). Groups from (5) to (9) received hyperlipidemic diet contained 10% (w/w) of wheat-germinated SWL formulae from (F3) to (F7).

Table 2. Constituents of the basal diet for 100 gm diet

Item	**%**
Corn starch	67.8
Casein	12.5
Corn oil	10.0
Vitamin mixture	1.0
Salt mixture	3.5
Cellulose	5.0
Choline chloride	0.2

Table 3. Constituents of vitamins mixtures used in the basal diet

Item	**Amount (g)**
Vitamin A palmitate 500.000 IU/gm	0.8
Vitamin D3 100.00 IU/gm	1
Vitamin E acetate 500 IU/gm	10
Menadione sodium bisulfite 62.,5% menadione	0.08
Biotin 1.0%	2
Cyano cobalaming 0.01%	1
Folic acid	0.2
Nicotinic acid	3
Calcium pantothenate	1.6
Pyridoxine HCl	0.7
Riboflavin	0.6
Thiamin-HCl	0.6
Sucrose	978.42

Table 4. Constituents of the hyperlipidemic diet for 100 g diet

Item	%
Corn starch	66.3
Casein	12.5
Animal fat	10
Cholesterol	1
Cholic acid	0.5
Vitamins mixture	1
Salt mixture	3.5
Cellulose	5
Choline chloride	0.2

2.6 Biochemical Measurements

2.6.1 Blood Sampling

At the end of the experiment, rats were fasted overnight and anesthetized (Youssef et al., 2014). Blood samples were collected from all animals from the retro-orbital plexus of each group into clean, dry and labeled tube. The tubes contained heparin (10.01 U/ml) as anticoagulant. Blood was centrifuged (3500 rpm for 15 min) to separate serum which was tightly kept in sealed aliquot tubes at -20 ºC until biochemical assay was carried out.

2.6.2 Determination of Total Triglycerides

Fully enzymatic determination of total triglycerides (TG) in serum was estimated spectrophotometrically at 546 nm according to the method of Wahlefeld (1974) of the enzymatic hydrolysis of triglycerides using kits followed by colorimetry determination of liberated glycerol.

2.6.3 Determination of Serum Total Cholesterol, LDL-c, VLDL-c and HDL-c

Enzymatic determination of total cholesterol (TC) in serum was carried out according to the method of Allian, Poon, Chan and Richmond (1974) using Stanbio kits (Texas, USA).

The determination of High Density Lipoprotein (HDL-c) was carried out according to Warnick, Benderson and Albers (1983) by the kits that were provided from Stanbio (Texas, USA). Low Density Lipoprotein (LDL-c) is precipitated from serum by magnesium chloride/dextrin sulfate reagent. HDL-c is then determined in supernatant using cholesterol reagent. LDL-c and VLDL-c were determined by the equations published by Friedewald, Levy and Fredrickson (1972).

2.7 Statistical Analysis

The values of measurements were expressed as means±standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey's *post hoc* test were performed to test the significance of differences ($P < 0.05$) between groups (Gustavo, José, Robison, Paulo, & José, 2012). The statistical analyses were carried out using Minitab software version 14.13 (USA).

3. Results and Discussion

3.1 Body Weight Gain, Food Intake and Food Efficiency Ratio

The results given in Table (5) revealed that the daily body weight gain (DBWG) and daily food intake (DFI) were positive in all studied groups (1-9) for the experimental rats. The rats of group fed on the hyperlipidemic diet (positive control) showed an increase in the DBWG compared to the rats of group fed on basal diet (negative control). Feeding on the diets enriched with biscuits composed of wheat flour and ungerminated/germinated sweet white lupin (SWL) oil and SWL flour decreased significantly ($P < 0.05$) the DBWG in the range from 1.28±0.30 g to 2.19±0.31 g compared to the positive control. The lowest value of DBWG was obtained with the rats of group (4) fed on hyperlipidemic diet contained 10% of ungerminated SWL biscuits formula F2. While, the highest value of DBWG was achieved within the rats of group (8) fed on hyperlipidemic diet contained 10% of germinated SWL formula F6 (25% oil and 35% flour). The value of DBWG in group 8 was very close to that of negative control (2.17±0.21). These results are in agreement with that of Atiat, Eman, Mona, and Ibrahim (1999), and Dadai, Walker, Sambrook, Welch and Owen (1996) who found a reduction of gain in body weight of

rats fed on legumes compared with hypercholesterolemic control. The decrease in final body weight may be due to the increase of legume protein intake and high fiber content that may cause weight loss (Anderson & Major, 2002). On the other hand, the DFI value (14.32±0.59 g) of positive control was markedly decreased ($P < 0.05$) compared with the negative control (15.92±0.55 g). This could be attributed to the presence of high amounts of fat (10%) and cholesterol (1%) in the hyperlipidemic diet. By enrichment of hyperlipidemic diet with 10% wheat biscuits and SWL biscuits (groups 3-9), the DFI amounts were increased more than that of the positive control. The highest value of DFI (15.42±0.5 g) was observed in group 9 of rats fed on hyperlipidemic diet contained 10% of germinated SWL formula F7 (25% oil and 40% flour) followed by group 8 (15.12±0.35 g) of rats fed on hyperlipidemic diet contained 10% of germinated SWL formula F6 (25% oil and 35% flour). The values of DFI in groups 8 and 9 are more in match with that of negative control (15.92±0.55) than others. This could be due to that germination could achieve desirable changes in nutritional characteristics of the SWL grains (Mousa, 2014). Furthermore, germination decreased the levels of anti-nutrients to improve the quality of SWL seeds. Food efficiency ratio (FER) was calculated by dividing DBWG on DFI (Sulieman & El-Newary, 2014). No significant difference ($P > 0.05$) of FER was noticed among groups, except groups 2 and 4 fed on hyperlipidemic diet and hyperlipidemic diet enriched with 10% ungerminated SWL biscuits. All these findings reflected the benefits of using germinated SWL oil and flour on the DBWG and DFI of induced hyperlipidemic rats.

Table 5. Effect of the studied groups on the body weight (g), food intake (g) and food efficiency of the experimental rats (induced hyperlipidemia)

Parameter	Starting weight (g)	DBWG (g)	DFI (g)	FER
Group 1 (BD)	109.53	2.17±0.21^{c}	15.92±0.55^{e}	0.14
Group 2 (HLD)	108.91	2.74±0.36^{d}	14.32±0.59^{a}	0.19
Group 3 (HLD+F1)	101.73	2.03±0.26^{c}	14.82±0.42^{b}	0.14
Group 4 (HLD+F2)	112.87	1.28±0.30^{a}	14.92±0.30^{b}	0.09
Group 5 (HLD+F3)	115.38	1.84±0.27^{b}	14.97±0.42^{b}	0.12
Group 6 (HLD+F4)	119.39	1.89±0.36^{b}	15.02±0.30^{b}	0.13
Group 7 (HLD+F5)	109.54	2.05±0.18^{c}	15.05±0.42^{b}	0.14
Group 8 (HLD+F6)	108.98	2.19±0.31^{c}	15.12±0.35^{c}	0.14
Group 9 (HLD+F7)	109.21	2.16±0.22^{c}	15.42±0.50^{d}	0.14

DBWG: Daily body weight gain; DFI: Daily food intake.

FER: Food efficiency ratio = DBWG/DFI (Sulieman & El-Newary, 2014).

BD: Basal diet; HD: Hyperlipidemic diet.

F1: Hyperlipidemic diet contained 10% (w/w) of wheat biscuits

F2: Hyperlipidemic diet contained 10% (w/w) of ungerminated SWL biscuits.

F3: Hyperlipidemic diet contained 10% (w/w) of germinated SWL (20% oil and 30% flour).

F4: Hyperlipidemic diet contained 10% (w/w) of germinated SWL (25% oil and 30% flour).

F5: Hyperlipidemic diet contained 10% (w/w) of germinated SWL (30% oil and 30% flour).

F6: Hyperlipidemic diet contained 10% (w/w) of germinated SWL (25% oil and 35% flour).

F7: Hyperlipidemic diet contained 10% (w/w) of germinated SWL (25% oil and 40% flour).

Values are expressed as mean±SD.

Values in each column which have different letters are significantly different at ($P < 0.05$).

3.2 Serum Lipid Profile

In the present study, the effect of short-term (6 weeks) consumption of hyperlipidemic diet supplemented with 10% ungerminated/germinated wheat-SWL biscuits and biscuits prepared from 100% refined wheat flour on lipid profile was studied and the results were presented in Tables 6 and 7.

The effects of feeding fat and cholesterol- enriched diet supplemented with different biscuit samples to the experimental rats for 6 weeks on the total cholesterol (TC) and triglycerides (TG) values are presented in Table 6. The results indicated a significant elevation ($P < 0.05$) in both TC and TG in hyperlipidemic diet group (positive control) as compared to negative control group (basal diet). Through the studied groups, data revealed that the reduction in TC and TG levels was obvious by the feeding of diets contained SWL biscuits (groups 4-9) compared to wheat biscuits (group 3). Furthermore, the reduction in TC and TG values was higher in formulated biscuits by germinated SWL oil and flour (group 5-9) than that of ungerminated SWL grains (group 4). The range of reduction in TC and TG levels was significantly ($P < 0.05$) changed by increasing the amounts of simultaneous substitution with germinated SWL oil and extracted flour in the formulated biscuits compared to control samples and wheat biscuits. The highest reductions of TC levels were observed in the groups 8 and 9 of rats fed on hyperlipidemic diet contained 10% of germinated SWL formula F6 (25% oil and 35% flour) and 10% of germinated SWL formula F7 (25% oil and 40% flour) as cited in Table 6. The values of TC in the groups of 8 and 9 were 169.80±0.34 and 168.10±0.16 mg/dL, respectively compared to 145.21±0.25 mg/dL of negative control and 405.16±0.32 of positive control. While, the lowest reductions in TC levels were achieved in the group of rats fed with wheat biscuits (208.10±0.15 mg/dL) and ungerminated SWL biscuits (198.80±0.18 mg/dL). The same findings were observed in the case of TG levels where the highest reductions were observed in the groups 8 and 9 of rats fed with germinated SWL formula F6 (91.23±0.35 mg/dL) and germinated SWL formula F7 (89.87±0.17 mg/dL) as compared with other examined groups. As well, the percent of decrements in groups 8 and 9 were 22.93% and 24.08%, respectively compared to the negative control. While, the lowest reductions were observed in the case of wheat biscuits (139.72±0.32 mg/dL) and ungerminated SWL formulae F2 (112.94±0.03 mg/dL). Therefore, the TC and TG values of the groups 8 and 9 were the best and all of them were cited in the normal ranges of TC (< 200 mg/dL) and TG (< 150 mg/dL). This substantial reduction in the values of TC and TG with SWL biscuits simultaneously substituted with germinated SWL oil and extracted flour after germination could be attributed to the combined effect of unsaturated fatty acids, dietary fiber and plant protein present in SWL seeds after germination. These results are in agreement with Jose et al. (2005) who reported that plasma total cholesterol concentration was decreased when rats were fed on lupin containing diet compared with control. Also, Fatima, Ann, Ian, Vernon and Robert (1996) and Mahfouz Elaby and Hassouna (2012) found that the mean plasma triacylglycerol level of positive control (hypercholesterolemic rats) was greater than that of rats fed on the legumes diets. The reduction of TG in the liver of rats using lupin protein has already been reported by Sirtori et al. (2004). It was attributed to the expression of the genes of the enzyme SREBP-1c, which is responsible for regulating the synthesis of fatty acids and triglycerides in the liver.

Table 6. Effect of the formulated biscuits on total cholesterol (TC) and triglycerides (TG) of the experimental rats (induced hyperlipidemia)

Groups	Total cholesterol (mg/dL) (Normal range < 200)	Triglycerides (mg/dL) (Normal range < 150)
Group 1 (-ve control)	145.21±0.25^{a}	118.37±0.08^{c}
Group 2 (+ve control)	405.16±0.32^{e}	183.72±0.21^{e}
Group 3	208.10±0.15^{d}	139.72±0.32^{d}
Group 4	198.80±0.18^{c}	112.94±0.03^{b}
Group 5	191.40±0.09^{c}	109.24±0.29^{b}
Group 6	190.50±0.11^{c}	110.83±0.14^{b}
Group 7	183.20±0.29^{c}	103.77±0.04^{b}
Group 8	169.80±0.34^{b}	91.23±0.35^{a}
Group 9	168.10±0.16^{b}	89.87±0.17^{a}

Values are expressed as mean±SD.

Values in each column which have different letters are significantly different at ($P < 0.05$).

The effect of feeding hyperlipidemic diet supplemented with different biscuits samples to the experimental rats for 6 weeks on low density lipoproteins cholesterol (LDL-c), very low density lipoproteins cholesterol (VLDL-c)

and high density lipoproteins cholesterol (HDL-c) are given in Table (7). The obtained results revealed significant decrease (P< 0.05) in HDL-c and significant increase (P < 0.05) in LDL-c and VLDL-c in hyperlipidemic diet group (positive control), as compared with negative control group. It could also be seen from the values cited in Table 7 that serum HDL-c concentration increased significantly (p < 0.05) in the groups of rats fed on hyperlipidemic diet contained 10% of germinated SWL formula F6 (25% oil and 35% flour) and 10% of germinated SWL formula F7 (25% oil and 40% flour). The values of HDL-c in the groups 8 and 9 were 70.43±0.11 and 71.51±0.19 mg/dL, respectively with high increments compared to the positive control (34.52±0.07 mg/dL) and very close to the negative control (72.21±0.13 mg/dL). While, the lower increments of HDL-c values were recorded with the rats fed with wheat biscuits (42.47±0.28 mg/dL) and ungerminated SWL biscuits F2 (49.73±0.03 mg/dL). On the other hand, the effect of studied groups on LDL-c and VLDL-c contents of the experimental rats (induced hyperlipidemia) was also studied. The LDL-c and VLDL-c values were calculated from the equations LDL-c = TC - (TG/5) – HDL and VLDL-c = TG/5 (Friedewald et al., 1972). The data revealed that all studied groups reduced LDL-c levels of the experimental rats in the range from 137.69±0.19 mg/dL (group 3) to 78.62±0.15 mg/dL (group 9). However, groups 8 and 9 recorded the highest decrement in LDL-c levels accounting to 81.12±0.09 and 78.62±0.15 mg/dL, respectively compared with the positive control (333.90±0.14 mg/dL). As well, the values of VLDL-c showed the highest significant decrements (P< 5) in the groups 8 and 9 of rats fed on hyperlipidemic- enriched diet with 10% of germinated SWL formula F6 (25% oil and 35% flour) and 10% of germinated SWL formula F7 (25% oil and 40% flour). Both of them recorded percent decrements VLDL-c of 22.90% in group 8 and 24.08% in group 9 compared to the negative control. In general, the values of HDL-c, LDL-c and VLDL-c in groups 8 and 9 were cited in the normal international ranges of > 45, < 100 and < 150 mg/dL, respectively.

Table 7. Effect of the formulated biscuits on serum lipoproteins of the experimental rats (induced hyperlipidemia)

Groups	HDL-c (mg/dL) (Normal range > 45)	LDL-c* (mg/dL) (Normal range < 100)	VLDL-c** (mg/dL) (Normal range < 150)
Group 1 (-ve control)	72.21±0.13^{d}	49.33±0.22^{a}	23.67±0.10^{d}
Group 2 (+ve control)	34.52±0.07^{a}	333.90±0.14^{f}	36.74±0.18^{f}
Group 3	42.47±0.28^{b}	137.69±0.19^{e}	27.94±0.05^{e}
Group 4	49.73±0.03^{b}	126.48±0.21^{e}	22.59±0.14^{c}
Group 5	60.44±0.13^{c}	109.12±0.25^{d}	21.84±0.26^{c}
Group 6	65.31±0.12^{c}	103.02±0.07^{d}	22.17±0.20^{c}
Group 7	69.32±0.23^{c}	93.13±0.26^{c}	20.75±0.19^{b}
Group 8	70.43±0.11^{d}	81.12±0.09^{b}	18.25±0.27^{a}
Group 9	71.51±0.19^{d}	78.62±0.15^{b}	17.97±0.14^{a}

* LDL-c = TC - (TG/5) – HDL ** VLDL-c = TG/5 (Friedewald et al., 1972).

Values are expressed as mean±SD.

Values in each column which have different letters are significantly different at (P < 0.05).

Therefore, the best values of all lipoprotein parameters TC, TG, HDL-c, LDL-c and VLDL-c were recorded with the SWL biscuits simultaneously substituted with germinated SWL oil (25%) and extracted flour (35% and 40%) after germination. It was appeared a significant improvement (P < 5) in all lipoprotein parameters by decreasing the values of LDL-c and VLDL-c as well as increasing the value of HDL-c compared to the positive control. The values of HDL-c, LDL-c and VLDL-c tended to match the control values in rat group fed on basal diet. The reason could be due to the effect of soluble dietary fiber, protein combined by unsaturated fatty acids that are improved after the germination process of SWL seeds. Huff and Telford (1985), Kingman (1991), and Andersson and Major (2002) reported that the LDL-c reduction observed by feeding legumes or their fractions to hypercholesterolemic subjects could result from reduced LDL synthesis and/or increased LDL metabolism. On the other hand, Sirtori et al. (2004) clearly indicated that protein from a naturally isoflavone-poor legume such as while lupin can effectively reduce cholesterolemia and, most likely, up regulate LDL receptor activity, a widely

accepted mechanism of cholesterol reduction associated with the intake of proteins. Moreover, El-Malky and Gouda (2007) indicated that germinated lupin seeds reduced the increase of serum glucose, TC, TG, LDL-c, VLDL-c and enhanced the increase of HDL-c. As well, Osman, Mahmoud, Romeilah and Fayed (2011) observed the supplementation of a hypercholesterolemia-induced diet with sweet lupin seeds significantly lowered the plasma levels of TC, TG and LDL-C. ALT, AST and LDH activities slightly decreased in treated groups compared with the hypercholesterolemic group (positive control).

3.3 Predictors of Coronary Risk

Total cholesterol/ High density lipoproteins cholesterol (TC/HDL-c), Low density lipoproteins cholesterol/High density lipoproteins-cholesterol (LDL/HDL-c; risk ratio; RR) and High density lipoproteins-cholesterol/Total cholesterol ratios (HTR%) are predictors of coronary risk (National cholesterol education program, 1994). The results presented in Table (8) indicated that the groups of rats fed on hyperlipidemic diet (positive control) showed significant increments ($P < 0.05$) in the values of RR and TC/HDL-c concurrent with a marked decrement in the value of HTR% compared to the rats fed with basal diet (negative control). It was obvious that the feeding with SWL biscuits supplemented with germinated SWL oil and extracted flour had significant lower ratios of TC/HDL-c and RR compared to the hyperlipidemic diet (positive control), wheat biscuits (group 3) and ungerminated SWL biscuits (group 4). The groups 8 and 9 of rats fed on hyperlipidemic- enriched diet with 10% of germinated SWL formula F6 (25% oil and 35% flour) and 10% of germinated SWL formula F7 (25% oil and 40% flour) showed TC/HDL-c ratios that characterized with low coronary risk (2.41±0.23 and 2.35±0.18) (Hassan et al., 2012). Meanwhile, feeding of wheat biscuits to rats resulted in a TC/HDL-c ratio which approached to the ratio that characterized with middle coronary risk (4.90±0.22). In the case of RR values, the feeding of SWL biscuits formulae F6 and F7 in the rats groups 8 and 9 recorded the lowest risk ratios of 1.15±0.10 and 1.10±0.17 with significant decrements ($P < 0.05$) compared with the induced hyperlipidemic rats (positive control) (9.67±0.11 RR). It could be concluded that, the effect of studied biscuits on TC/HDL-c and RR in descending order was wheat biscuits (group 3) > ungerminated SWL biscuits group (4) > germinated SWL biscuits (20% oil and 30% flour) > germinated SWL biscuits (25% oil and 30% flour) > germinated SWL biscuits (30% oil and 30% flour) > germinated SWL biscuits (25% oil and 35% flour) > germinated SWL biscuits (25% oil and 40% flour). The TC/HDL-c and RR of groups 8 and 9 were tended to match that of the negative control. Furthermore, the group 9 of rats fed on biscuits formula F7 exhibited maximum increase in HTR% (42.54%) followed by the group 8 of rats fed on biscuits formula F7 (41.48%) as could be seen in Table 8. Whereas, the groups 3 and 4 of rats fed on wheat biscuits and ungerminated SWL biscuits showed the lowest increase in the HTR (20.41% and 25.02%). It could be seen from the obtained results that adding germinated SWL oil and extracted flour enriched biscuits to the diet could produce significant improvement for the beneficial lipoprotein ratios to reduce the risk of heart disease in hyperlipidemic individuals. Gustavo, José, Robison, Paulo and José (2012) demonstrated that protein isolate from *Lupinus albus* has a metabolic effect on endogenous cholesterol metabolism and a protector effect on the development of hepatic steatosis. Whereas, El-Malky and Gouda (2007) indicated that germinated lupin seeds reduced the hepatic enzymes ALT and AST and avoided the damage in liver tissues resulted from hyperlipidemic diet effect compared to the positive control.

Atherogenic index (AI) indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidney (Hassan et al., 2012). The higher the AI, the higher is the risk of above organs for oxidative damage (Basu et al., 2007). Atherogenic lipoprotein profile of plasma is an important risk factor for coronary artery disease. The results cited in Table 8 revealed that the rats of group 9 that fed on hyperlipidemic-enriched diet with 10% of germinated SWL formula F7 (25% oil and 40% flour) showed a maximum lower AI value (1.35) followed by the group 8 of rats fed on hyperlipidemic- enriched diet with 10% of germinated SWL formula F6 (25% oil and 35% flour) with 1.41 as compared to the positive control (10.74). These results are in accordance with those obtained by Marchesi et al. (2008) who studied the hypolipidemic and anti-atherogenic effect of lupin protein isolates in rabbits and reported a significant reduction of cholesterol and a reduction of the risk of developing atherosclerosis. Furthermore, Mahfouz et al. (2012) recommended to utilize white lupin to prepare healthy diets to protect against hypercholesterolemia.

Table 8. Predictors for coronary risk

Groups	RR	TC/HDL-c	HTR%	AI
Group 1 (-ve control)	0.68±0.18	2.01±0.19	49.73	1.01
Group 2 (+ve control)	9.67±0.11	11.74±0.20	8.52	10.74
Group 3	3.24±0.24	4.90±0.22	20.41	3.90
Group 4	2.54±0.12	3.99±0.10	25.02	2.99
Group 5	1.81±0.19	3.17±0.11	31.58	2.17
Group 6	1.58±0.09	2.92±0.12	34.28	1.92
Group 7	1.34±0.25	2.64±0.25	37.84	1.64
Group 8	1.15±0.10	2.41±0.23	41.48	1.41
Group 9	1.10±0.17	2.35±0.18	42.54	1.35

RR: risk ratio= LDL-c/HDL-c.

HTR: High density lipoproteins-cholesterol/ Total cholesterol ratio = (HDL-c / TC) × 100.

AI: Atherogenic index = (TC - HDL-c) / HDL-c.

4. Conclusion

In the present study, it was observed that feeding of high cholesterol and fat diets supplemented with functional sweet white lupin (SWL) biscuits to the hyperlipidemic rats resulted in a significant decrease ($P < 0.05$) in the values of TC, TG, LDL-c and VLDL-c concurrent with a significant increase ($P < 0.05$) in the value of HDL-c compared with wheat biscuits. In addition, the feeding of hyperlipidemic diet contained 10% of functional SWL biscuits reduced markedly the predictors for the coronary heart risk such as RR, AI and TC/HDL-c compared to the group of rats fed on hyperlipidemic diet (positive control). Furthermore, it was found that the enrichment of hyperlipidemic diets with germinated SWL oil and extracted flour improved markedly the depression of serum cholesterol and food efficiency compared to the ungerminated ones. The acceptable results of these biological parameters were achieved by the simultaneous substitution with 25% germinated SWL flour and 35% or 40% germinated SWL oil. Therefore, it could be suggested that the proposed functional SWL biscuits were able to regulate the blood cholesterol and the body growth levels of individuals and patients. The modification in the lipid profile and food efficiency of induced hyperlipidemia rats caused by the feeding of functional SWL biscuits may have some physiological importance and may be easily extrapolated to humans.

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