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IMPACT OF FEEDING BREAD ENRICHED WITH FLAXSEED ON PLASMA PROFILE OF HYPERLIPIDEMIC RATS – A SHORT REPORT

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The present research was postulated to study the possibility of producing bread containing either the whole flaxseed or the defatted flaxseed and to determine to what extent it may modify lipid profile in hyperlipidemic rats. The results showed the reduction in plasma total lipids, total cholesterol, low density lipoprotein cholesterol and triglycerides when rats were fed different flaxseed-containing bread. Concomitantly, bread containing the whole flaxseed produced a significant increase in high density lipoprotein cholesterol. These results reflect the possible beneficial use of such bread towards cardiovascular diseases.

INTRODUCTION

There is a considerable interest in the potential health benefits of oil seeds, especially regarding cardiovascular disease and cancer. This interest in oil seeds relates to their high content of polyunsaturated fatty acids, particularly α-linolenic acid (ALA) [Conquer & Holub, 1996], vegetable protein [Kritchevsky, 1995], soluble fiber [Anderson *et al.*, 1990], flavonoids and related compounds [Setchell, 1995], which may possess antioxidant [Hertog *et al.*, 1993] and sex hormone agonistic activities [Collins *et al.*, 1997].

Flaxseed (linseed) is the small flat oval seed from flax *Linum usitatissimum* L., Family Linaceae [Oomah & Mazza, 2000]. It dispalys potential beneficial effects towards immune function [Kelley *et al.*, 1991], inflammatory diseases [Mohamed *et al.*, 2003] and may have beneficial effects concerning the prevention of platelet aggregation [Bierenbaum *et al.*, 1993]. Flaxseed consumption in various forms as a food ingredient and for its medicinal properties dates from 500 BC since its cultivation [Oomah & Mazza, 1998]. It is therefore not surprising that flaxseed is the most prominent oilseed studied to date as a functional food, since it is a leading source of ALA (52% of total fatty acids) and of phenolic compounds known as lignans [Oomah & Mazza, 2000]. These and other components of flaxseed incorporation in the diet are particularly attractive for the development of foods with specific health advantages.

The objective of the present study was to prepare a bread supplemented with the whole or defatted flaxseed as a functional food for hyperlipidemic subjects. The study addressed also the evaluation of the hypolipidemic activity of the prepared functional food in hypercholesterolemic rats.

MATERIALS AND METHODS

MATERIALS

Flaxseed Giza 8 variety was obtained from the Field Crops Research Institute, Agriculture Research Centre.

Bread ingredients. White American flour (72% extraction), fine granulated sugars, common salt, bakery shortening, instant dry yeast and skimmed milk.

Animals. White male albino rats of 115–122 g body weight were used in the study. The animals were kept individually in wire bottomed cages at room temperature. Water and food were given *ad-libitum*.

METHODS

Flaxseed preparation. A part of flaxseed was ground and defatted with petroleum ether (40–60°C) using a Soxhlet apparatus.

Optimization of bread formulation. Control bread (CB) was made according to the method of Lagrange [1995]. Flaxseed was ground and added as the whole or defatted seeds at levels of 15% and 10%, respectively, on the expense of flour, to make two samples of bread.

Chemical analysis of bread samples. All the bread samples were dried, powdered and sieved through 100-mesh sieve. All the dried samples were analyzed for moisture, protein, fat, crude fiber and ash contents using standard AOAC procedure [AOAC, 1995].

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Preparation of diets. Different experimental diets, presented in Table 1, were used in the study. A salt mixture and vitamin mixtures were prepared according to Briggs and Williams [1963] and Morcos [1967], respectively. Oil soluble vitamins were given orally at a dose of 0.1 mL/rat per week.

Sensory evaluation. The baked bread samples were cooled to room temperature and subjected to sensory analysis by 10 panelists [Meiselman, 1978]. Each panelist was served a control bread sample along with the test samples and was asked to assign scores on a nine-point scale for appearance, crumb colour, texture, taste and overall acceptability. A sensory score of 5 or above was deemed acceptable, and a sensory score below 5 was considered unacceptable.

DESIGN OF ANIMAL EXPERIMENT

First stage. Rats were assigned to two dietary groups. The first group (6 rats) received a balanced diet, while thirty animals (the second group) were fed a hypercholesterolemic diet reported by Zulet *et al.* [1999]. This stage continued for 26 days.

Second stage. After the development of hypercholesterolemia, hypercholesterolemic rats (the second stage) were divided into five sub-groups each of 6 rats. Rats of the first sub-group were continuously administered with the same hypercholesterolemic diet (HH group) and the remaining hypercholesterolemic sub-groups of rats received a balanced diet (HB group), a diet containing the control bread (CB) or the bread supplemented with the whole or defatted flaxseed for four weeks (for diets' composition see Table 1). During the experimental period, the control group (the first stage) was continuously administered with the same balanced diet (CC group).

During the experiment, body weight and food intake were recorded twice a week. At the end of the first and second stages, the total food intake, body weight gain and food efficiency ratio (body weight gain/total food intake) were calculated. Blood samples were collected from all rats after an overnight fast at the end of the first and second stages for the determination of plasma total lipids [Toro & Ackerman, 1975], total cholesterol (T-ch) [Watson, 1960], high density lipoprotein cholesterol (HDL-ch) [Burstein *et al.*, 1980], low density lipoprotein cholesterol (LDL-ch) [Gerard & Gerald, 1981] and triglycerides (TG) [Megraw *et al.*, 1979]. An HDL-ch / T-ch ratio was calculated as well.

The results of animal experiment were expressed as the mean±SE and they were analyzed statistically using the Student's t-test. The results of a sensory evaluation were represented as mean±SE and were evaluated statistically using the one-way analysis of variance ANOVA followed by the Duncan's test. In all cases, $p<0.05$ was used as the criterion of statistical significance.

TABLE 1. Composition of different experimental diets (g per 100 g).

Ingredients	Diets				
	Bread diets[2]				
	Balanced	Hypercholesterolemic	CB	WG8	DFG8
Casein	11.9*	11.9*	-	-	-
Corn oil	10	-	7.3	4.7	7.7
Coconut oil	-	25	-	-	-
Sucrose	19.5	35	19.5	19.5	19.5
Starch	49.1	17.6	-	19.49	15.95
Salt mix.	3.5	3.5	3.5	3.5	3.5
Vit. mix.	1	1	1	1	1
Fiber	5	5	5	4.91	4.95
Cholesterol	-	1	-	-	-
Dry bread samples[1]	-	-	63.7	46.9	47.4

* 11.9 casein has been shown to contain 10 g protein [AOAC, 1995]; [1]An appropriate amount of different dried bread samples was added to the different diets so as each diet would contain 10% protein, 10% fat, 19.5% sucrose, 5% fiber, 1 vitamin mix. 3.5% salt mix. and completed to 100% by starch; [2]CB: Control bread, WG8: Bread contain Giza 8, DFG8: Bread contains defatted Giza 8.

RESULTS AND DISCUSSION

Fresh bread samples when dried appeared to demonstrate more or less the same moisture content (27–28 %).

The proximate composition of dry bread samples showed that flaxseed bread samples were rich in protein compared to the control bread. The addition of the whole or defatted flaxseed powder to flour elevated protein content of the bread samples from 15.7% in the CB bread to 21.3% and 21.1% in the whole or defatted flaxseed bread, respectively. In our previous study, it has been shown that flaxseed was rich in protein (23.2%) and fat (38.3%) [Mohamed, in press]. Bread samples containing the whole flaxseed demonstrated the highest content of fat 11.4%, whereas the control bread showed the lowest content of fat 4.2%. The fat content of defatted flaxseed bread was 4.9%. The high fat content of the whole flaxseed bread samples was due to the high content of fat in flaxseed. Flaxseed is the richest plant source of ALA [Oomah & Mazza, 2000]. Ash content was 0.8, 1.4 and 1.3% in control, the whole flaxseed and defatted flaxseed bread, respectively. The content of crude fiber was very low in all bread samples, reaching 0.19% in the whole flaxseed bread, 0.11 in defatted flaxseed bread, and 0.0 in control bread.

The sensory evaluation of bread samples revealed that flaxseed bread samples were accepted by the panelist. The appearance of control bread, the whole flaxseed bread and defatted flaxseed bread was scored as 8.5±0.167, 8.2±0.133 and 8.0±0.0; texture as: 8.2±0.133, 7.9±0.1 and 7.8±0.133; taste as: 8.5±0.167, 7.8±0.133 and 7.7±0.153, and overall acceptability as: 8.1±0.1, 7.6±0.178, 7.7±0.153, respectively. There was no significant difference in the appearance, texture, taste and overall acceptability of flaxseed bread compared to the control bread. However crumb colour score was significantly higher in the control bread sample (8.6±0.163) compared with the defatted flaxseed bread (6.4±0.221) and the whole flaxseed bread (6.7±0.153) ($p<0.05$), which is due to the dark colour attained by flaxseed. No significant differences were detected between flaxseed bread samples in all sensory parameters.

Plasma lipid profiles of hypercholesterolemic rats (first stage) are shown in Table 2. The rats fed the hypercholesterolemic diet showed a significant increase in the plasma

levels of total lipids (+145%, p<0.001), total cholesterol. (+124%, p<0.001), and LDL-cholesterol (+556%, p<0.001), which was accompanied by a decrease in HDL-ch and HDL/T-ch ratio (-42 %, -74% respectively, p<0.001) when compared to normal rats. Plasma TG level showed a non-significant change. These results are in agreement with the results of Zulet *et al.* [1999] who reported a significant increase in plasma levels of T-ch and LDL-ch (+362% and +2660%, p<0.001, respectively) of rats fed a similar hypercholesterolemic diet.

In our study, hypercholesterolemia was induced in rats by feeding them a diet rich in coconut oil (25%), 35% sucrose, 1% cholesterol and devoid of fibers, which is similar to a diet employed by different authors in previous studies [Zulet *et al.*, 1996, 1999]. These authors have used a diet containing 25% coconut oil, 48.4% sucrose, 1% cholesterol and 0.5% cholic acid. The assessment of the lipid profile in plasma of rats fed a high-fat diet enriched in saturated fat and cholesterol revealed the incidence of hypercholesterolemia, which was accompanied by a decrease in HDL-ch and an increase in LDL-ch. These alterations resembled a situation of type II a hyperlipidemia in humans [Tholstrup *et al.*, 1995], which could be associated with a down-regulation in LDL receptors by the cholesterol and saturated fatty acids included in the diet [Stucchi *et al.*, 1995].

Nutritional parameters of normal and hypercholesterolemic rats of the first stage are shown in Table 2. The results revealed that non-significant changes were found in final body weight and body weight gain, whereas the total food intake and food intake/day were significantly lower in hypercholesterolemic rats in comparison with the normal rats (p<0.025 and p<0.010, respectively).

Plasma lipids of hypercholesterolemic rats before (B) and after feeding (A) different dietary treatments in the second stage are shown in Table 3. A statistical analysis of different plasma lipids indicated insignificant differences between different hypercholesterolemic groups at the start before any dietary treatment (B). This pointed to the reliability of the results when comparing plasma lipids of different groups at the end of the experiment (A).

When comparing plasma lipids of different hypercholesterolemic rats fed balanced diets containing different flaxseed bread with rats continuously fed the hypercholesterolemic diet (HH), as it was expected all plasma lipids

TABLE 2. Plasma lipid profile and nutritional parameters of normal and hypercholesterolemic rats (first stage).

Parameters	Groups	
	Normal Mean ± SE	Hypercholesterolemic Mean ± SE
Total lipids (g/dL)	0.315 ± 0.015	0.773*** ± 0.008
% Change		+ 145
Tch (mg/dL)	76.3 ± 1.829	171.0*** ± 2.152
% Change		+ 124
HDL-ch (mg/dL)	39.8 ± 0.833	23.0*** ± 0.316
% Change		- 42
HDL-ch/Tch ratio	0.522 ± 0.005	0.136 ± 0.002
% Change		- 74
LDL-ch (mg/dL)	19.3 ± 1.085	126.6*** ± 2.024
% Change		+ 556
TG (mg/dL)	80.5 ± 1.477	82.3 ± 0.753
% Change		+ 2
Initial body weight (g)	118.8 ± 1.077	118.7 ± 2.544
Final body weight (g)	156.3 ± 2.333	156.2 ± 2.666
Body weight gain (g)	37.5 ± 1.309	37.3 ± 1.563
Total food intake (g)	343.5 ± 4.239	328.3* ± 5.407
Food intake (g/day)	13.21 ± 0.163	12.6** ± 0.154
Food efficiency ratio	0.109 ± 0.003	0.113 ± 0.0096

Values significantly differ from normal rats: *: p<0.025, **: p<0.010, ***: p<0.001.

TABLE 3. Plasma lipids of hypercholesterolemic rats fed different experimental diets (second stage).

Parameters		Groups											
		CC		HH		HB		CB		WG8		DFG8	
		B	A	B	A	B	A	B	A	B	A	B	A
Total lipids	Mean	0.315	0.313	0.754	0.866*	0.765	0.683•	0.768	0.654•	0.788	0.529•c	0.769	0.557•c
(g/dL)	±SE	0.015	0.007	0.024	0.032	0.019	0.02	0.019	0.02	0.027	0.026	0.017	0.012
	%Change				+177		-21		-4		-23		-18
T.Ch (mg/Dl)	Mean	76.3	76.8	171.2	197.2*	179.3	153.5•	164.7	133.5•	174.5	112.8•c	168.9	129.1•a
	±SE	1.829	0.949	6.407	8.462	5.461	7.153	7.051	7.587	7.877	5.557	3.763	3.219
	%Change				+157		-22		-13		-27		-16
HDL-Ch	Mean	39.8	39.83	22.3	20.3*	23.3	28.7•	22.2	28•	25.3	32.3•b	23.8	28.7
(mg/dL)	±SE	0.833	0.167	0.615	0.422	0.667	0.715	0.601	0.516	0.954	0.843	1.014	0.989
	%Change				-49		+41		-2		+13		0
HDL-Ch/T.Ch	Mean	0.522	0.515	0.131	0.104*	0.131	0.188•	0.136	0.21•	0.147	0.291•c	0.141	0.223•a
ratio	±SE	0.005	0.004	0.005	0.006	0.003	0.008	0.009	0.019	0.008	0.018	0.006	0.009
	%Change				-80		+81		+12		+55		+19
LDL-Ch.	Mean	19.3	19.7	126.7	157.8*	131.5	104.8•	123.8	91•	129.3	62.8•c	124	81.8•a
(mg/Dl)	±SE	1.085	0.954	5.839	7.823	4.848	6.476	7.585	10.518	7.635	5.46	3.66	3.369
	%Change				+701		-34		-13		-40		-22
TG (mg/dL)	Mean	80.5	80.7	80.8	79.8	84.3	84.5	80.2	80.7	81.7	76a	83.2	79.7c
	±SE	1.477	0.715	3.359	2.329	0.803	1.118	1.621	1.333	3.039	2.943	0.703	0.558
	%Change				-1		+6		-4		-10		-6

Values significantly differ from normal rats:*: p< 0.001. Values significantly differ from HH rats: * p < 0.001. Values significantly differ from HB rats: a: p<0.010, b: p<0.005, c: p<0.001. CB: Control bread, WG8: Bread contain Giza 8, DFG8: Bread contains defatted Giza 8.

were significantly improved. To exclude variation of plasma lipids that may occur as a result of changing the diet from the hypercholesterolemic to balanced one, we decided to compare different groups of rats fed bread or flaxseed bread containing diets with the group of hypercholesterolemic rats fed the balanced diet (HB) to know the actual change in plasma lipids. Hypercholesterolemic rats fed the balanced diet (HB group) showed a significant reduction in plasma levels of total lipids, T-ch and LDL-ch (-21%, -22%,-34%, respectively, $p<0.001$), whereas their HDL-ch and HDL-ch/T-ch ratio increased significantly (+41%, +81%, respectively, $p<0.001$), in comparison to the hypercholesterolemic rats that continued feeding the hypercholesterolemic diet (HH).

Hypercholesterolemic rats fed the balanced diet containing free flaxseed bread showed non-significant changes in plasma lipid profile, while feeding rats the balanced diet containing bread supplemented with the whole or defatted flaxseed produced significant changes (improvements) in plasma lipid profile with variable degrees compared with hypercholesterolemic rats fed the balanced diet (HB group). Feeding hypercholesterolemic rats a diet containing bread supplemented with the whole or defatted flaxseed showed a significant reduction in plasma level of triglycerides by 10 and 6%, respectively.

The reduction in plasma levels of T-ch and LDL-ch in hypercholesterolemic rats fed a balanced diet containing bread supplemented with the whole or defatted flaxseed in our study is in agreement with the results of Bierenbaum *et al.* [1993]. Previous studies showed that the consumption of flaxseed either raw or defatted produced a reduction in the total and LDL-cholesterol in humans [Clark *et al.*, 1995; Jenkins *et al.*, 1999]. These studies stimulated us to fortify bread with flaxseed to be used as hypolipidemic functional food.

The hypolipidemic effect of flaxseed may be ascribed to the presence of lignans, vegetable protein, and α-linolenic acid and its seed coat gum. This gum is a highly viscous mixture of acidic and neutral polysaccharides that have been characterized as glucuronic acids, rhamnose, arabinose, xylose and galactose [Oomah & Mazza, 1995]. The polysaccharide gum makes up to 8% of the whole flaxseed [Oomah & Mazza, 1995]. Flaxseed gum has been used in the treatment of hyperglycemia and hypercholesterolemia in humans [Oomah & Mazza, 2000]. The reduction in LDL-ch in our study may be related to an increase in bile acid synthesis [Marlett *et al.*, 1994] with consecutive greater fecal losses of bile acid [Miettinen & Tarpila, 1977].

Plant proteins have been shown previously to dispaly hypocholesterolemic activity [Atwal *et al.*, 1997]. The major protein isolated from flaxseed has been shown to be characterized by high contents of the amino acids arginine, glutamate/glutamine, and aspartate/asparagine [Chung, 2001]. Food sources rich in arginine have been reported to have potential preventative functions against heart disease [Pszczola, 2000].

Flaxseed is a rich source of lignans, with potential weak estrogenic activity [Hertog *et al.*, 1993]. Lignans may block androgen or progesterone receptors, thereby may alter cardiovascular disease risk by changing HDL-cholesterol metabolism [Thompson *et al.*, 1989].

Flaxseed is rich in ALA [Oomah & Mazza, 2000] which is an ω-3 fatty acid and has been reported to be useful in the prevention and treatment of coronary artery disease and hypertension [Simopoulos, 1999]. This is because ALA is the precursor for the synthesis of eicosapentaenoic and docosahexaenoic acids which are associated with the control of cardiovascular diseases [Bibus *et al.*, 1998]. ALA also reduces serum triglycerides and the development of thrombosis and arteriosclerosis [Pszczola, 1998].

Nutritional parameters of hypercholesterolemic rats after feeding different dietary treatments (stage 2) are shown in Table 4. No significant changes were observed in all nutritional parameters of rats subjected to different dietary treatments.

CONCLUSION

Breads supplemented with the whole or defatted flaxseed showed significant improvements in plasma lipid profile of hypercholesterolemic rats and were acceptable from the sensory point of view. Therefore such bread can be recommended as functional food to treat hypercholesterolemia or reduce the risk of atherosclerosis.

REFERENCES

1. Anderson J.W., Deakins D.A., Floore T.L., Smith B.M., Whitis S.E., Dietary fiber and coronary heart disease. Crit. Rev. Food Sci. Nutr., 1990, 29, 95–110.
2. AOAC., Official Methods of Analysis of the Association of Official Analytical Chemists, 1995, 12th ed Washington DC.
3. Atwal A.S., Kubow C., Wolynetz M.S., Effect of protein source and amino acid supplementation on plasma cholesterol in guinea pig. Int. J. Vitam. Nutr. Res., 1997, 67, 192–198.

TABLE 4. Nutritional parameters of different experimental groups (second stage).

	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Total food intake (g)	Food intake (g/day)	Food efficiency
CC	156.3 ± 2.333	194.2 ± 3.533	37.8 ± 1.939	338.3 ± 6.539	12.1 ± 0.234	0.112 ± 0.005
HH	153.5 ± 3.584	194 ± 3.558	40.5 ± 0.764	335 ± 4.281	11.9 ± 0.153	0.122 ± 0.003
HB	157.8 ± 6.669	201.3 ± 8.047	43.5 ± 2.029	343.3 ± 8.026	12.3 ± 0.288	0.127 ± 0.003
CB	161.8 ± 10.142	204 ± 9.318	42.2 ± 2.007	341.7 ± 6.665	12.2 ± 0.248	0.123 ± 0.004
WG8	158.5 ± 8.765	204 ± 7.177	45.5 ± 2.667	340 ± 4.281	12.1 ± 0.156	0.135 ± 0.007
DFG8	158.2 ± 5.114	200.2 ± 5.769	42 ± 1.769	339.5 ± 3.095	12.1 ± 0.105	0.125 ± 0.003

CB: Control bread, WG8: Bread contain Giza 8, DFG8: Bread contains defatted Giza 8.

4. Bibus D.M., Stitt P., Holman R.T., Ratios of linoleic to α-linolenic acids in the diet: Implications in the health of humans and companion animals. 1998: *in*: Proceedings of the 57th Flax Institute of the U. S., Fargo, ND, 26–28 March 1998, pp. 49–52.
5. Bierenbaum M.L., Reichstein R., Watkins T.R., Reducing atherogenic risk in hyperlipemic humans with flaxseed supplementation: a preliminary report. J. Am. Coll. Nutr., 1993, 12, 501.
6. Briggs G.M., Williams M.A., A new mineral mixture for experimental rat diets and evaluation of other mineral mixtures. Fed. Proc., 1963, 22, 261.
7. Burstein M., Scholnick H.R., Morfin R., Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. Scand. J. Clin. Lab. Invest., 1980, 40, 583.
8. Chung M.W.Y., Isolation and characterization of the major fraction of flaxseed proteins. 2001, MSc. Thesis, The University of British Columbia, Vancouver, BC, Canada, pp 25–32.
9. Clark W.F., Parbtani A., Huff M.W., Spanner E., Salis H.D., Chin-Yee I., Philbrick D.J., Holub B.J., Flaxseed: a potential treatment for lupus nephritis. Kidney Int., 1995, 48, 475–483.
10. Collins B.M., McLachlan J.A., Arnold S.F., The esterogenic and antiesterogenic activities of phytochemicals with the human esterogen receptor expressed in yeast. Steroids, 1997, 62, 365–370.
11. Conquer J.A., Holub B.J., Supplementation with an algae source of docosahexaenoic acid increases (n-3) fatty acid status and alters selected risk factors for heart disease in vegetarian subjects. J. Nutr., 1996, 126, 3032.
12. Gerard T., Gerald A.L., Process and reagents for the selective separation of low density lipoprotein (LDL) and for the quantification of their components. Eur. Path. (Appl.) E.P., 1981, 76, 211–223.
13. Hertog M.G., Feskens E.J., Hollman P.C., Katan M.B., Kromhout D., Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet, 1993, 342, 1007–1012.
14. Jenkins D.G., Kendall C.W., Vidgen E., Agarwal S., Rao A.V., Rasenberg R.S., Diamandis E.P., Novokmet R., Mechling C.C., Perera T., Griffin L.C., Cunnane S.C., Health aspects of partially defatted flaxseed, including effects on serum lipids, oxidative measure and *ex vivo* androgen and progestin activity: a controlled cross-ovar trial. Am. J. Clin. Nutr., 1999, 69, 395–403.
15. Kelley D.S., Branch L.B., Love J.E., Taylor P.C., Rivera Y.M., Iacono J.M., Dietary alphaa-linolenic acid and immunocompetence in humans. Am. J. Clin. Nutr., 1991, 53, 40–49.
16. Kritchevsky D., Dietary protein, cholesterol and atherosclerosis: a review of the early history. J. Nutr., 1995, 125 (suppl), 589S–596S.
17. Lagrange V., Flaxseed in bakery products. Technical Bulletin. Flax Council of Canada, 1995, 38, 1–6.
18. Marlett J.A., Hosig K.B., Vollendrof N.W., Mechanism of serum cholesterol reduction by oat bran. Hepatology, 1994, 20, 1450–1458.
19. Megraw R., Dunn D., Biggs H., Mannual and continuous flow colorimetry of triglycerols by a fully enzymatic method. Clin. Chem., 1979, 25, 273–284.
20. Meiselman H.L., Scales for measuring food preferences. 1978, *in*: Encyclopedia of Food Sciences (eds. M.S. Petersen, A.H. Johnson). AVI, Westport, CT, pp. 675–688.
21. Miettinen T.A., Tarpila S., Effect of pectin on serum cholesterol, fecal bile acids and biliary lipids in normolipidemic and hyperlipidemic individuals. Clin. Chim. Acta, 1977, 79, 471–479.
22. Mohamed D.A., Ismael A.I., Ibrahim H.A., Evaluation of the anti-inflammatory and biochemical effect of flaxseed oil in inflamed rats. Kasr EL Aini Med. J., 2003, 9, 79–96.
23. Mohamed D.A, Hypotriglyceridemic effect of flaxseed in rats. Kasr EL Aini Med. J., 2005 (in press).
24. Morcos S.R., The effect of protein value of the diet on the neurological manifestations produced in rats by β-immodipropionitrile. Br. J. Nutr., 1967, 21, 269–273.
25. Oomah B.D., Mazza G., Flaxseed dietary fiber and cyanogens. 1995, *in*: Flaxseed in Human Nutrition (eds. Cunnane S.C, Thompson L.U). Champaign, IL: AOCS Press, pp. 56–67.
26. Oomah B.D., Mazza G., Flaxseed products for disease prevention in functional foods. 1998, *in*: Biochemical and Processing Aspects (ed. G. Mazza). Technomic Publishing, Lancaster, PA, pp. 91–99.
27. Oomah B.D., Mazza G., Bioactive components of flaxseed: occurrence and health benefits. 2000, *in*: Phytochemicals and Phytopharmaceuticals. (eds. F. Shahidi, C.T. Ho.), Champaign: AAOCS Press, pp. 105-112.
28. Pszczola D.E., The ABCs of nutritional ingredients. Food Tech., 1998, 52 , 30–38.
29. Pszczola D.E., Genes and diet: the specialized role ingredients may play. Food Tech., 2000, 54, 82–88.
30. Setchell K.D., Discovery and importance of lignans. 1995, *in*: Flaxseed in Human Nutrition (eds. S.C. Cunnane, LU. Thompson). Champaign, IL: AOCS Press, pp. 82–93.
31. Simopoulos A.P., Essential fatty acids in health and chronic disease. Am. J. Clin. Nutr., 1999, 70 (3 Suppl), 560S–568S.
32. Stucchi A.F., Terpstra A.H.M., Nicolosi R.J., LDL receptor activity is down-regulated similarly by a cholesterol-containing diet high in palmitic acid or high in lauric and myristic acids in cynomolgus monkeys. J. Nutr., 1995, 125, 2055–2067.
33. Tholstrup T., Marckmann P., Vessby B., Sandstrom B., Effect of fats high in individual saturated fatty acids on plasma lipoprotein a levels in young healthy men. J. Lipid Res., 1995, 36, 1447–1454.
34. Thompson P.D., Cullinane E.M., Sady S.P., Contrasting effects of testosterone and stanozolol on serum lipoprotein levels. JAMA. 1989, 261, 1165–1173.
35. Toro G., Ackerman P.G., Practical Clinical Chemistry, 1975, 1st edition, Little, Brown and Company, Boston USA, pp. 352–359.
36. Watson D., A simple method for the determination of serum cholesterol. Clin. Chem. Acta., 1960, 5, 637–642.
37. Zulet M.A., Diez A., Barber A., Fernandez-Otero M.P., Martinez J.A., Carbohydrate digestion and absorption in a dietary-induced model of hypercholesterolemia. J. Physiol., 1996, 493, 102S–108S.
38. Zulet M.A., Macarulla M.T., Portillo M.P., Noel-Sube-

rville C., Higueret P., Matinez J.A., Lipid and glucose utilization in hypercholesterolemic rats fed a diet containing heated chickpea (*Cicer Aretinum* L.): a potential functional food. Int. J. Vitam. Nutr. Res., 1999, 69, 403–409.

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