https://doi.org/10.46389/rjd-2024-1441
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2024; volume 31, issue 2, pages 143–149

Original Article

Postprandial changes in gene expression of hepatic cholesterol metabolism in response to two protein sources in the rat

Amine Allaoui [1, 2] *, Faiza Dehiba [1, 3], Luis Vicente Herrera-Marcos [4, 5], Maria-Jesus Rodríguez-Yoldi [6], Jesus Osada [4], Ahmed Boualga [1]

[1] Laboratory of Clinical and Metabolic Nutrition, Department of Biology, Faculty of the Nature and Life Sciences, University of Oran1, Oran, Algeria

[2] Laboratory of Eco-Biology and Environmental Protection, Department of Biology, Faculty of the Nature and Life Sciences, University of Blida1, Blida, Algeria

[3] Department of Biology, Higher School of Biological Sciences of Oran, Oran, Algeria

[4] Department of Biochemistry and Molecular and Cellular Biology, Veterinary Faculty, University of Zaragoza, CIBERobn (ISCIII), IIS Aragón, IA2, Zaragoza, Spain

[5] INSERM, IMRB, Health Faculty, University of Paris-Est Créteil, Créteil, France

[6] Departament of Pharmacology and Physiology, Legal and Forensic Medicine, Physiology Unit, Veterinary Faculty, University of Zaragoza, CIBERobn (ISCIII), IIS Aragón, IA2, Zaragoza, Spain

* Correspondence to: Amine Allaoui, Laboratory of Clinical and Metabolic Nutrition, Department of Biology, Faculty of Nature and Life Sciences, University of Oran1, Oran, Algeria. E-mail: allaoui_amine@univ-blida.dz

Received: 13 January 2024 / Accepted: 11 April 2024

Abstract

A hypocholesterolemic effect of chickpea (CPH) and sardine protein hydrolysates (SPH) has been observed. Two main mechanisms could be involved in explaining this fact: the inhibition of cholesterol enterohepatic cycle and a post-absorptive regulatory pathway. We aimed to check whether these hypotheses were involved in the present study. Three groups of rats were given a single dose of a cholesterol-oil solution (HC), supplemented or not with CPH or SPH. The postprandial transcription levels of some genes involved in cholesterol metabolism were assessed in their livers. Four hours after feeding, the results showed that Mttp, Pltp, Cidec, Abca1, and Abcg1 gene expressions were similar among the different groups. Lcat mRNA was 5.5-fold higher in CPH and SPH rats' liver *vs.* HC, but this difference was not statistically significant. SPH tends to upregulate Ldlr expression, while CPH tends to upregulate Cyp7a1 transcription (2- and 8-fold, respectively, compared to HC rats, p=0.083 according to the Mann-Whitney test). Pon1 and Apoa1 were not affected by the hydrolysate supplementation *vs.* HC group. In conclusion, these results suggest that chickpeas and sardine protein hydrolysates exert hypocholesterolemic activity mainly by enterohepatic inhibiting the cholesterol cycle rather than modulating the postprandial gene expressions involved in cholesterol metabolism.

Keywords: hypocholesterolemia, chickpea, sardine, protein hydrolysate, rats, postprandial.

Abbreviations: Abca1 and Abcg1 – ATP-binding cassette A1 and G1; C – control; Cidec – cell death inducing DFFA like effector C; CPH – chickpea protein hydrolysate; Cyp7a1 – cholesterol 7 alpha-hydroxylase; HC – high-cholesterol; Lcat – lecithin-cholesterol acyltransferase; Ldlr – low density-lipoprotein receptor; Lxr – liver X receptor; M±SD – means±standard deviation; Mttp – microsomal triglyceride transfer protein; Pltp – phospholipid-transfer protein; Pon1 – paraoxonase; qPCR – quantitative polymerase chain reaction; SPH – sardine protein hydrolysate.

Introduction

The impact of food proteins, in particular their hydrolysates, on lipid homeostasis is now well established. Indeed, they are no longer considered for their nutritional value only, but depending on their sources and preparation method, they could exhibit beneficial health properties [1–3]. However, the mechanism by which they could exert their bioactivities is still being debated. It was suggested that dietary proteins modulate cholesterol metabolism through two mechanisms. One involves the enterohepatic cycle, which inhibits cholesterol absorption and the induction of its excretion, while the other concerns the role played by certain amino acids and peptides in hormonal signaling and constitutes a post-absorptive regulatory pathway [4–7].

Many works were undertaken in our laboratory to investigate whether and how fish and legume proteins could modulate cholesterol metabolism [8–12]. In our last works [13–15], chickpeas and sardine protein hydrolysates were tested on hypercholesterolemic rats, and a hypocholesterolemic effect was observed for both ingredients. Several mechanisms might be involved in explaining this bioactivity. It could be that the two hydrolysates would promote apo-B lipoproteins clearance from the bloodstream via the activation of low-density lipoproteins receptor (LDLR), activate cholesterol elimination via its conversion to bile acids by activating the cholesterol 7 alpha-hydroxylase (CYP7A1), inhibit its absorption in the gut by decreasing its micellar solubility, and enhance HDL functionality by increasing lecithin-cholesterol acyltransferase (LCAT) and paraoxonase (PON1) activities. Hence, this study was conducted to verify these hypotheses to check for possible postprandial gene transcription changes following chickpea and sardine protein hydrolysates supplementation in hypercholesterolemic rats.

Material and methods

Protein hydrolysate preparations

Sardine (*Sardina pilchardus*) and chickpea (*Cicer arietinum*) protein isolate preparation were previously described by Benomar *et al.* [15] and Yahia *et al.* [14], respectively. The crude protein contents were between 87 and 90 percent as determined according to the AOAC method number 984.13 [16]. The obtained protein isolates were hydrolyzed with Alcalase 2.4L (Novozyme, Denmark) under the same conditions: pH 8.0; 50°C, and enzyme: substrate ratio of 3 U/mg. The hydrolysis reaction was followed using the pH-stat method [17], and the mixture pH was continuously adjusted with a 4 M NaOH solution. The reaction was stopped at a degree of hydrolysis of 8% by heating the mixture at 90°C for 10 min. The protein hydrolysates were centrifuged at 5000×g for 20 min, and the soluble phase was lyophilized (Christ, ALPHA 1-2 model, Germany) for the present study. Sardine (SPH) and chickpea (CPH) protein hydrolysates amino acids composition are presented in Table 1.

Animal treatment

Twelve male Wistar rats, randomized into four groups of 3 each, were housed in cages in a room maintained at 22°C with a 12h cycle of light and dark. Rats were kept according to the general guidelines on using living animals in scientific investigation [18]. Throughout the acclimatization period, the animals were given free access to tap water and standard chow.

On the day of the experiment, the rats (125±15 g) were fasted for 16 hours and then received, per gavage, 1.5 ml of a commercial oil (80% soy oil and 20%

Table 1: The amino acid composition of chickpea and sardine proteins hydrolysates.

%	SPH	CPH
Isoleucine	4.1±0.0	4.9±0.1
Leucine	8.7±0.1	9.3±0.2
Valine	7.1±0.1	5.6±0.0
Methionine	3.4±0.0	1.2±0.1
Alanine	9.2±0.0	7.2±0.0
Tyrosine	3.1±0.0	1.9±0.0
Histidine	11.0±0.1	3.6±0.4
Tryptophane	5.9±0.1	0.7±0.1
Phenylalanine	12.7±0.0	4.5±0.2
Alanine	9.2±0.0	7.2±0.0
Glycine	8.4±0.1	5.2±0.2
Glutamate	8.9±0.1	10.9±0.6
Serine	3.9±0.0	7.3±0.2
Arginine	5.9±0.1	8.2±0.1
Lysine	8,5±0.9	8.6±0.2
Taurine	3.9±0.1	-

Note: SPH – sardine protein hydrolysate; CPH – chickpea protein hydrolysate.

https://doi.org/10.46389/rjd-2024-1441

sunflower oil, Cevital, Bejaia, Algeria) mixed with one of the following:

- 75 mg of cholesterol (Sigma-Aldrich Chemie, Germany) (HC group);
- 75 mg of cholesterol + 200 mg of chickpeas hydrolysate (CPH group);
- 75 mg of cholesterol + 200 mg of sardine hydrolysate (SPH group);
- A fasting group, without any supply, was considered as control (C).

The rats were anesthetized and sacrificed four hours after gavage. A piece (200 mg) of the same liver lobe was excised and then immersed in 5 volumes of RNALater® (Sigma-Aldrich, MO, USA). Samples were incubated at 4˚C overnight and then transferred to—20˚C until their RNA extraction.

RNA extraction and purification

Total RNA was extracted and purified from 20 mg of tissues using a Quick-RNATM MiniPrep kit (Zymo Research, CA, USA) following manufacturer instructions. The RNA concentration and purity were checked by measuring absorbance at 280, 260, and 230 nm in a SPECTROstar Nano (BMG Labtech, Germany).

To check the RNA integrity, samples were run on 1% agarose gel electrophoresis stained with ethidium-bromide. The gel was then exposed to UV light, and images were captured (BioRad, Madrid, Spain) to check for the presence and intensity of 28S and 18S ribosomal RNA bands. Equal amounts of DNA-free RNA from each sample were used in reverse transcription and quantitative polymerase chain reaction (qPCR) analysis.

RNA reverse transcription

The mRNA was reverse-transcribed using the Thermo Scientific™ First Strand cDNA Synthesis kit (CA, USA) in a PTC-100 TM Thermal Cycler (Bio-Rad, CA, USA). The samples were then heated to 70˚C for 5 minutes, and the DNA was stored at -40˚C.

Gene expression level analysis

Gene expression was analyzed on StepOnePlus Real-Time PCR (Applied Biosystems, USA). According to the manufacturer's instructions, qPCR reactions were performed using Power SYBR Green (Applied Biosystems, CA, USA). Peptidyl-prolyl cis-trans isomerase B (Ppib) gene was used to normalize gene expression. Primers (Table 2) were designed and checked by BLAST analysis (NCBI) to verify the specificity and selective amplification of the target gene, and the specificity of the PCR reaction was confirmed by observing

Table 2: Primers sequences.

Gene symbol	Accession	Sequence
Abca1	NM_178095	Sense: TCGGCTGGTATCGATTTCACA Antisense: GGTCCCAGTACCCATCCTTGAT
Abcg1	NM_053502	Sense: TCTGACCTTTCCCCTCGAGAT Antisense: AGTACACGATGCTGCAGTAGGC
Apoa1	NM_012738	Sense: GGCAGAGACTATGTGTCCCAGTTT Antisense: TTGAACCCAGAGTGTCCCAGTT
Cidec	NM_001024333	Sense: TCACTGTCCAGGCATGTAGCA Antisense: CCTTTGCGAACCTTCCGAT
Cyp7a1	NM_012942.2	Sense: CATTACAGAGTGCTGGCCAAGA Antisense: CTGTCCGGATATTCAAGGATGC
Lcat	NM_017024	Sense: GGCTGTGCTACCGAAAGACAGA Antisense: GACAACCCTGGTGTTATCAATCCA
Ldlr	NM_175762.3	Sense: GACTGCAAGGACAAGTCGGA Antisense: GCACTGGGTCACATTGATGC
Mttp	NM_001107727.1	Sense: GAAAAATCGGGTGGCTGTGG Antisense: GGGTACTGGGAGAACTGCAC
Pltp	NM_001168543	Sense: GTTGAATGAGCGTATCTGGCGT Antisense: CAACAGTGACGAAGCCTGCAT
Pon1	NM_032077	Sense: GGACTGGTGTTGGCACTTTACA Antisense: CACCCGCTTCGATTCCTTTA

a single dissociation curve. Each primer concentration was adjusted for a PCR efficiency between 90 and 110%. The relative amounts of mRNAs were calculated by the $2^{(-\Delta\Delta CT)}$ method. Each sample was performed in duplicate.

Statistical analysis

Values are expressed as means±standard deviation (M±SD). Statistical analysis was performed using the Kruskal Wallis-ANOVA non-parametric test compared to the HC group. Values were considered significant at $p<0.05$.

Results and discussion

Hepatic Lcat gene expression was approximately 5.5-fold increased in CPH and SPH rats *vs*. HC (Table 3), even though the difference was not statistically significant (p=0.100). This was concomitant with our previous finding that both hydrolysates, given to hypercholesterolemic rats for a month, did not enhance LCAT activity [13]. Bettzieche *et al*. [7] also reported no effect of lupin protein on this gene. In contrast, Shukla *et al*. [6] noticed an Lcat gene up-regulation after fish protein consumption.

According to Table 3, liver Mttp, Pltp, and Cidec gene expressions did not differ in treated groups compared to HC, suggesting that the tested products did not increase the rate of lipoprotein synthesis or storage. Abca1 and Abcg1 transcripts did not show a difference among groups either.

Cholesterol 7 alpha-hydroxylase gene transcription was respectively 2.5- and 8.4-times increased in SPH and CPH *vs*. HC liver, whereas the statistical difference could not be found (p=0.054). Shukla *et al*. [6] reported that gene expression of Cyp7a1 did not differ after fish protein feeding. Hosomi *et al*. [4] reported that fish protein hydrolysate supply stimulates the hepatic activity of cholesterol 7 alpha-hydroxylase compared to a C group (without cholesterol). Whereas, compared to HC rats, the fish protein group did not show a significant difference in liver Cyp7a1 gene expression, which confirms our findings (Figure 1). Same findings were also reported with soy proteins compared to HC rats [19].

In our previous study, the cholesterolemia was decreased in HC rats given SPH and CPH for a month, and by analyzing the present findings, the hypocholesterolemic mechanism could not be explained by the postprandial modulation of Cyp7a1 gene expression. Indeed, according to Zhao, Wright [20], and Staels, Fonseca [21], the up-regulation of Cyp7a1 is mainly a result of long-term liver cholesterol accumulation. The long-term hypocholesterolemic property of SPH and CPH found by Dehiba *et al*. [13] could then be attributed to the ability of some hydrophobic peptides to inhibit the cholesterol micellar solubility and their higher binding capacity with bile acids [22] conducting possibly to the increase in bile acids synthesis. This was also confirmed by the high fecal cholesterol contents in [14, 15].

It was suggested that the high contents of taurine and/or glycine in fish and chickpeas could play an important role in bile acid secretion [12, 14], enhancing, then, a fall in cholesterol liver and serum levels. This leads to a decrease in the level of hepatic cholesterol, thus promoting an increase in LDLR activity, which purifies cholesterol from the bloodstream. In this work,

Table 3: Postprandial liver gene expression levels (arbitrary unit) with no significant differences between rats in response to different protein supply.

	HC	CPH	SPH	C	Kruskal-Wallis (p-values)
Lcat	1.2±0.6	5.7±0.9	6.3±0.3	3.6±1.3	0.100
Mttp	1.0±0.2	1.7±0.3	2.2±1.4	1.5±0.3	0.186
Cidec	1.0±0.1	2.3±2.0	2.7±1.4	0.9±0.2	0.230
Pltp	1.0±0.4	1.5±1.0	2.9±0.4	1.2±0.5	0.152
Abca1	1.8±2.1	1.3±0.9	0.6±0.2	0.7±0.3	0.637
Abcg1	1.0±0.3	1.4±0.8	1.3±0.2	0.8±0.1	0.624

Note: Values are means±standard deviation. HC – high cholesterol group; SPH – sardine protein hydrolysate group; CPH – chickpea protein hydrolysate group; C – control; Lcat – lecithin-cholesterol acyltransferase; Mttp – microsomal triglyceride transfer protein; Cidec – cell death inducing DFFA like effector C; Pltp – phospholipid-transfer protein; abca1 and Abcg1 – ATP-binding cassette A1 and G1.

https://doi.org/10.46389/rjd-2024-1441

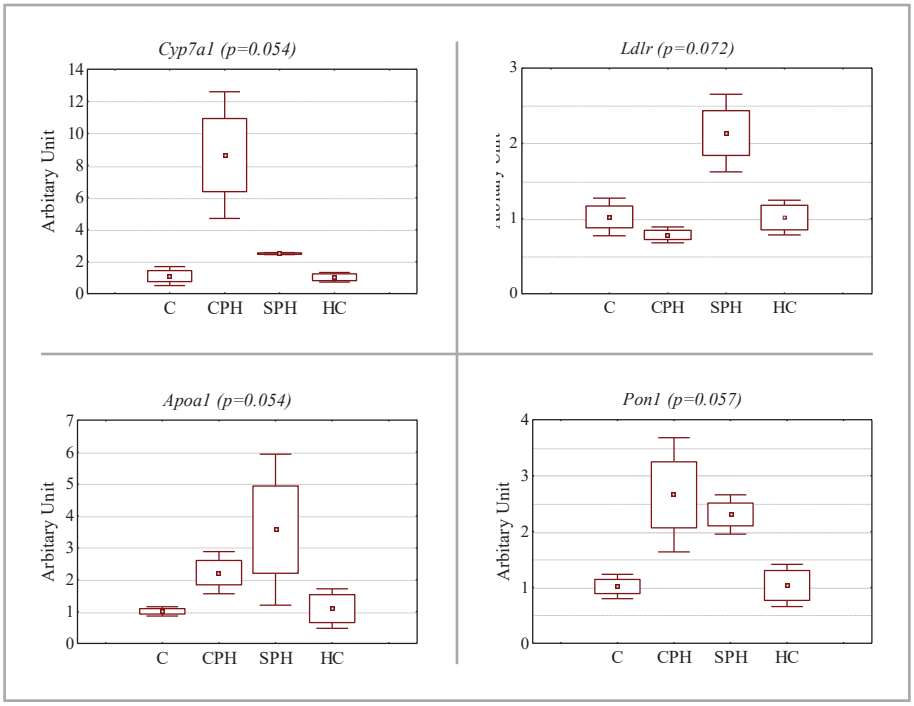

Figure 1: Postprandial Lxrα, Cyp7a1, Ldlr, Apoa1, and Pon1 gene expression levels in response to sardine and chickpea protein hydrolysates supplementation. ⊡ – Mean; ▭ – Mean±SEM; ⊥ – Mean±SD. The p-values were estimated according to the Kruskal-Wallis test. HC – high cholesterol group; SPH – sardine protein hydrolysate group; CPH – chickpea protein hydrolysate group; C – control; Lxr – liver X receptor; Cyp7a1 – cholesterol 7 alpha-hydroxylase; Ldlr – low density-lipoprotein receptor; Pon1 – paraoxonase.

the liver contents of Ldlr mRNA were 2 times increased in the SPH group *vs*. HC (Figure 1) but with no statistical significance. Hosomi *et al*. [4] reported that dietary fish hydrolysate did not influence the liver gene expression levels of Ldlr. But, in another work, Shukla *et al*. [6] found that the liver of rats fed fish protein expressed higher relative Ldlr mRNA concentrations than in rats fed casein. Here again, this effect could be explained by the long-term diet supply and not by the postprandial regulation of fish protein.

CPH did not, nor did it modulate Ldlr expression. Bettzieche *et al*. [7] reached the same results using lupin protein, where they remarked no significant alteration in Ldlr gene transcription, as well as Lcat and Cyp7a1. Contrarily, in hepatic cell culture, Cho *et al*. [23] and Lammi *et al*. [24] pointed out an increase in Ldlr transcription levels by soy and lupine protein hydrolysates, respectively. Peptide transformation in the gastrointestinal tract could be the explanation for the discrepancy in results. At the same time, it is difficult to compare these studies since they were not carried out under hypercholesterolemic or the same physiologic conditions.

We also reported in [13] that chickpea and sardine hydrolysates enhanced paraoxonase activity in HC rats. However, the increase in paraoxonase gene expression in CPH and SPH rat livers, compared to the HC group, was not significant (>2.2-fold; p=0.057). Apoa1 mRNA, an activator of PON1 [25], was also found to increase after an SPH and CPH supplementation (>2-times; p=0.054), compared to HC rats (Figure 1). This disagree with Shukla *et al*. [6] results. In the studies of Yahia *et al*. [14] and Benomar *et al*. [15], the serum APOA1 contents did not differ in HC rats after SPH and CPH supplementation. However, according to Dehiba *et al*. [13], PON1 activity was enhanced in SPH and CPH groups *vs*. HC. This could confirm a non-postprandial regulatory effect of both hydrolysates.

Conclusion

Since SPH and CPH did not significantly change the expression of the genes involved in cholesterol metabolism, it could be concluded that their hypocholesterolemic property is mainly due to a gastrointestinal pathway inhibiting the enterohepatic cholesterol cycle. However, we cannot exclude the fact that these two nutraceuticals may also modulate the expression of the cholesterol metabolism genes, but this could be possible after a long-term administration of the protein hydrolysates.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Approval

The local Committee of Animal Care and Use approved the animal study protocol (SAF 2016-75441-R).

Funding

This research was financially supported by the National Fund for Scientific Research and Technological Development of the Algerian Ministry of Higher Education and Scientific Research.

References

1. Nasri M. Protein hydrolysates and biopeptides: Production, biological activities, and applications in foods and health benefits. A review. In: Advances in Food and Nutrition Research, Toldrá F (ed). Academic Press, pp 109-159, 2017. doi:10.1016/bs.afnr.2016.10.003
2. Agyei D, Tan K-X, Pan S, Udenigwe CC, Danquah MK. Peptides for biopharmaceutical applications. In: Peptide Applications in Biomedicine, Biotechnology and Bioengineering, Koutsopoulos S (ed). Woodhead Publishing, pp 231-251, 2018. doi:10.1016/B978-0-08-100736-5.00009-0
3. Cicero AFG, Fogacci F, Colletti A. Potential role of bioactive peptides in prevention and treatment of chronic diseases: a narrative review. Br J Pharmacol 174 (11):1378-1394, 2017. doi:10.1111/bph.13608
4. Hosomi R, Fukunaga K, Arai H, Kanda S, Nishiyama T, Yoshida M. Fish protein hydrolysates affect cholesterol metabolism in rats fed non-cholesterol and high-cholesterol diets. J Med Food 15 (3):299-306, 2012. doi:10.1089/jmf.2011.1620
5. Liaset B, Madsen L, Hao Q *et al.* Fish protein hydrolysate elevates plasma bile acids and reduces visceral adipose tissue mass in rats. Biochim Biophys Acta 1791 (4):254-262, 2009. doi:10.1016/j.bbalip.2009.01.016
6. Shukla A, Bettzieche A, Hirche F, Brandsch C, Stangl GI, Eder K. Dietary fish protein alters blood lipid concentrations and hepatic genes involved in cholesterol homeostasis in the rat model. Br J Nutr 96 (4):674-682, 2006. doi:10.1079/bjn20061895
7. Bettzieche A, Brandsch C, Weiße K, Hirche F, Eder K, Stang GI. Lupin protein influences the expression of hepatic genes involved in fatty acid synthesis and triacylglycerol hydrolysis of adult rats. Br J Nutr 99:952-962, 2008. doi:10.1017/S0007114507857266
8. Athmani N, Dehiba F, Allaoui A *et al.* Sardina pilchardus and Sardinella aurita protein hydrolysates reduce cholesterolemia and oxidative stress in rat fed high cholesterol diet. J Exp Integr Med 5 (1):47-54, 2015. doi:10.5455/jeim.180115.or.119
9. Allaoui A, Barranquero C, Yahia S *et al.* Fenugreek proteins and their hydrolysates prevent hypercholesterolemia and enhance the HDL antioxidant properties in rats. Nutr Food Sci 48 (6):973-989, 2018. doi:10.1108/NFS-02-2018-0062
10. Boualga A, Prost J, Taleb-Senouci D *et al.* Purified chickpea or lentil proteins impair very-low-density lipoprotein (VLDL) metabolism and lipoprotein lipase activity in epididymal fat, but not in muscle, compared to casein, in growing rats. Eur J Nutr 48 (3):162-169, 2009. doi:10.1007/s00394-009-0777-4
11. Bouderbala S, Lamri-Senhadji MY, Boualga A, Bouchenak M, Belleville J. Reverse cholesterol transport in hypercholesterolemia rats fed differents proteins and lipids origins. Nutr Food Sci 39:128-134, 2009. doi:10.1108/00346650910943235
12. Affane F, Louala S, el Imane Harrat N *et al.* Hypolipidemic, antioxidant and antiatherogenic property of sardine by-products proteins in high-fat diet induced obese rats. Life Sci 199:16-22, 2018. doi:10.1016/j.lfs.2018.03.001
13. Dehiba F, Allaoui A, Benomar S *et al.* Protective properties of sardine and chickpea protein hydrolysates against lipoprotein oxidative damages and some inflammation markers in hypercholesterolemic rats. Med J Nutrition Metab 14 (4):439-452, 2021. doi:10.3233/mnm-210548
14. Yahia S, Benomar S, Dehiba F *et al.* Hypocholesterolemic and antioxidant efficiency of chickpea (Cicer arietinum) protein hydrolysates depend on its degree of hydrolysis in cholesterol-fed rat. Nutr Food Sci 47 (2):254-269, 2017. doi:10.1108/NFS-04-2016-0046
15. Benomar S, Yahia S, Dehiba F *et al.* Differential antioxidative and hypocholesterolemic responses to two fish protein hydrolysates (Sardina pilchardus and Boops boops) in cholesterol-fed rats. Nutr Food Sci 45 (3):448-466, 2015. doi:10.1108/NFS-04-2016-0046
16. Horwitz W. Food composition; additives; natural contaminants. In: Official methods of analysis of AOAC International, Horwitz W, AOAC International (eds). vol 2. 17th edn. AOAC International, Gaithersburg, 2000.
17. Adler-Nissen J. A review of food hydrolysis specific areas. In: Enzymic Hydrolysis of Food Proteins, Adler-Nissen J (ed). Elsevier Applied Science Publishers, Copenhagen, pp 57-109, 1986.
18. Council of European Communities. Council instructions about the protection of living animals used in scientific investigations. Official Journal of the European Communities (JO 86/609/CEE) L358, Brussels, 1987.
19. Arellano-Martínez GL, Granados O, Palacios-González B, Torres N, Medina-Vera I, Tovar AR. Soya protein stimulates bile acid excretion by the liver and intestine through direct and indirect pathways influenced by the presence of dietary cholesterol. Br J Nutr 111 (12):2059-2066, 2014. doi:10.1017/s0007114514000361
20. Zhao C, Wright KD. Liver X receptor in cholesterol metabolism. J Endocrinol 204:233-240, 2010. doi:10.1677/JOE-09-0271
21. Staels B, Fonseca VA. Bile acids and metabolic regulation: mechanisms and clinical responses to bile acid sequestration. Diabetes Care 32 (suppl_2):S237-S245, 2009. doi:10.2337/dc09-S355
22. Ruiz-Ruiz JC, Betancur-Ancona DA, Segura-Campos MR. Bioactive vegetable proteins and peptides in lipid-lowering; nutraceutical potential. Nutr Hosp 29 (4):776-784, 2014. doi:10.3305/nh.2014.29.4.7208
23. Cho SJ, Juillerat MA, Lee CH. Cholesterol lowering mechanism of soybean protein hydrolysate. J Agric Food Chem 55 (26):10599-10604, 2007. doi:10.1021/jf071903f

 https://doi.org/10.46389/rjd-2024-1441

24. Lammi C, Zanoni C, Scigliuolo GM, D'Amato A, Arnoldi A. Lupin peptides lower low-density lipoprotein (LDL) cholesterol through an up-regulation of the LDL receptor/sterol regulatory element binding protein 2 (SREBP2) pathway at HepG2 cell line. J Agric Food Chem 62 (29):7151-7159, 2014. doi:10.1021/jf500795b

25. Viktorinova A, Jurkovicova I, Fabryova L *et al*. Abnormalities in the relationship of paraoxonase 1 with HDL and apolipoprotein A1 and their possible connection to HDL dysfunctionality in type 2 diabetes. Diabetes Res Clin Pract 140:174-182, 2018. doi:10.1016/j.diabres.2018.03.055