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Fenugreek proteins and their hydrolysates prevent hypercholesterolemia and enhance the HDL antioxidant properties in rats

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Abstract

Purpose – This paper aims to investigate the *in vivo* hypocholesterolemic property of fenugreek proteins (FP), Purafect-fenugreek protein hydrolysate (PFPH) and Esperase-fenugreek protein hydrolysate (EFPH) on high cholesterol (HC)-fed rats.

Design/methodology/approach – Rats were randomized into five groups: four were fed for four weeks a hypercholesterolemic diet and the tested products were given by gavage. The fifth group was taken as control (C) receiving the same diet without cholesterol.

Findings – Results showed that the elevated aspartate aminotransferase activity in HC group plasma was significantly corrected by FP and EFPH administration (−33 per cent; $p = 0.0003$). HC liver lipids and total cholesterol (TC) contents were not markedly affected by FP and EFPH. However, liver triglycerides (TG) contents trended to decrease in FP rats vs HC ($p = 0.07$), while, the TG decrease was significant in groups fed the proteins hydrolysates ($p = 0.02$). On the other hand, serum TC and TG decreased by 53 per cent ($p = 0.0003$) and 20 per cent ($p = 0.04$), respectively, in FP treated rats compared to HC group. This decrease was associated with a high fecal cholesterol excretion (2.5-fold higher in FP vs HC; $p = 0.0001$). Likewise, EFPH-treated rats exhibited lower TC compared to HC rats ($p = 0.004$). The very low density lipoprotins was the main affected fraction in these two groups, while there were no significant difference in apolipoproteins (Apo) B, A-I and A-IV contents between the different groups, except in FP group, where Apo A-I and A-IV decreased by 26 and 17 per cent, respectively, compared to C rats ($p = 0.02$). The high density lipoproteins (HDL) of rats treated with proteins hydrolysates showed a better antioxidant property compared to those of HC rats, which was accompanied with an increase in paraoxonase activity when compared to HC group.

Originality/value – Unlike PFPH which had almost no effect, FPs and EFPH could constitute a nutraceutical ingredient in cardiovascular disease management.

Keywords Fenugreek, Hypercholesterolemia, HDL antioxidants properties, Lipoproteins, Protein hydrolysates

Paper type Research paper

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1. Introduction

Hypercholesterolemia and oxidative stress is one of the leading causes of atherosclerosis and cardiovascular diseases (CVD). These two factors are often observed in the modern life style. As consequence of the world life transition, CVD, and especially dyslipidemia, had immensely increased either in occidental societies or in emerging countries, reaching a prevalence of 53 per cent in the USA, 50.5 per cent in Spain and 48 per cent in France (with 30 per cent for hypercholesterolemia) (Ferrières *et al.*, 2009; Guallar-Castillón *et al.*, 2012; Tóth *et al.*, 2012). In Algeria, the prevalence of dyslipidemia is less than 15 per cent as reported in "Epidemiological Transition and Health Impact in North Africa (TAHINA) Survey 2002" (Institut National de Santé Publique, 2007).

It is generally established that the nutritive and non-nutritive constituents of food can be used to prevent the risk of developing or complicating human disease conditions (Bouchenak and Lamri-Senhadji, 2013). Fenugreek (*Trigonella foenum-graecum*) is a leguminous used as food and spice for seasoning (Srinivasan, 2013). It is equally used in Indian and Chinese traditional medicine for the treatment of many diseases (Bukhari *et al.*, 2008), as well as in the Mediterranean countries, Western countries, Asia and Africa. The seeds are the most important and useful part of this plant (Meghwal and Goswami, 2012). Many of the functional and medicinal properties of fenugreek are attributed to its chemical composition (20-25 per cent protein, 45-50 per cent dietary fiber, 20-25 per cent mucilaginous soluble fiber, 6-8 per cent fatty acids and essential oils and 2-5 per cent steroidal saponins) (Khorshidian *et al.*, 2016). Most of these constituents have been investigated in recent studies (Mowla *et al.*, 2009; Madhava Naidu *et al.*, 2011; Reddy and Srinivasan, 2011; Elmnan *et al.*, 2012; Belguith-Hadriche *et al.*, 2013; Habib-Martin *et al.*, 2017). To the best of our knowledge, although these studies clearly demonstrated the beneficial role of non-protein components such as saponine, fiber, flavonoid and trigoneline on lipid metabolism and redox status (El Khoury and Anderson, 2013), no comparative study has been conducted on the role of fenugreek proteins (FP) or their hydrolysates on hypercholesterolemia. Indeed, fenugreek is a rich protein legume and because of its high protein content and its favorable amino acid composition, the seeds could be a potential source of bioactive peptides (Christiane *et al.*, 2009).

Indeed, in addition to their nutritional function, proteins can also be considered as bioactive molecules providing other functional and nutraceutical properties (Garcia *et al.*, 2013; López-Barrios *et al.*, 2014). In this regard, administration of proteins from different sources have shown promising results in improving the quality of life, particularly in CVD and dyslipidemia prevention (Möller *et al.*, 2008; Ruiz *et al.*, 2014; Udenigwe and Rouvinen-Watt, 2015). In fact, the action of proteins is because of their nature and their amino acid composition. In addition, more recent studies have shown that the proteins may also contain biologically active peptide sequences which, after their release during gastrointestinal digestion, are responsible in part of their properties. Moreover, it is believed that some of these peptides exhibit the same properties of the protein that contained them but with greater potential or even exhibit new properties. According to researchers, the release of these bioactive peptides from their proteins is also possible by controlled hydrolysis *in vitro* (Sarmadi and Ismail, 2010; Lemes *et al.*, 2016; Cicero *et al.*, 2017; Nasri, 2017).

The main biological cardioprotective activities attributed to bioactive peptides to date are antioxidant, hypolipidemic, antithrombotic and antihypertensive (Sarmadi and Ismail, 2010; Cicero *et al.*, 2017). The exact mechanism by which proteins decreased cholesterolemia is not fully understood. In this sense, it has been reported that protein hydrolysates and peptides exhibited hypocholesterolemic activity via bile acid binding and disruption of cholesterol micelles in the gastrointestinal tract, and/or by altering hepatic and adipocytic enzymes

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activities and gene expression of lipogenic proteins, which can modulate aberrant physiological lipid profiles (Udenigwe and Howard, 2013).

In the present study, the hypocholesterolemic and antioxidant properties of FG proteins and their hydrolysates obtained by using two commercial enzymes (Purafect or Esperase) were investigated in rats that were fed hypercholesterolemic diet. Fenugreek was chosen for its high proteins content and its favorable amino acid composition.

2. Material and methods

2.1 Preparation of fenugreek proteins

Fenugreek was purchased from a local spices market in the city of Tiaret (Algeria). Seeds were then cleaned, grounded to a fine powder and defatted in Soxhlet (Labotech LT-6, Rosdorf, Germany) by using *n*-hexane for 12 cycles (10 h) and the FP were purified according to Boye *et al.* (2010) method. The defatted powder was solubilized with distilled water in a ratio of 1:20 (w/v) and the pH was adjusted to 10 with 1N NaOH solution. After 30 min, the preparation was centrifuged (Refrigerated Centrifuge Sigma DE) at 2,000 *g* for 30 min at 4°C and the resulting supernatant was brought to isoelectric point (pH 4.5) with 2N HCl. Proteins were removed after centrifugation (3,000 *g* for 30 min) and rinsed twice with distilled water. The purified proteins isolate was freeze dried and stored at 4°C. The nitrogen content in FP isolate was determined according to the AOAC method number 984.13 (Horwitz, 2000), and crude protein, estimated by multiplying total nitrogen content by the factor of 6.25. FP isolate contained 80.3 per cent of total proteins.

2.2 Preparation of fenugreek protein hydrolysates

FPs were hydrolyzed by using Esperase® (pH 9.0; 50°C) or Purafect® 2000E (pH 10.0; 50°C) both from Sigma Chemical Co. (St. Louis, MO, USA). FPs isolate was dissolved in distilled water at a proportion of 5 per cent (w/v). The pH and temperature were then adjusted to optimum enzyme activity prior to their addition. The enzyme/protein ratio was fixed at 5 (U/mg of protein). Once the enzyme was added, the pH of the solution was maintained constant by a continuous addition of 2N NaOH solution. The degree of hydrolysis of FP was monitored using a pH-stat method (Adler-Nissen, 1986).

$$\mathrm{DH}(\%) = \frac{\mathrm{h}}{\mathrm{h_{tot}}} \times 100 = \frac{\mathrm{B} \times \mathrm{N_B}}{\mathrm{MP}} \times \frac{1}{\alpha} \times \frac{1}{\mathrm{h_{tot}}} \times 100$$

where B is the amount (mL) of NaOH consumed to keep the pH constant during the reaction, N_B is the normality of NaOH, MP is the mass of proteins (g) and α is the average degree of dissociation of the α-NH_2 groups released during hydrolysis. The total number of peptide bonds (h_{tot}) was assumed to be 7.6 meq/g.

When the degree of hydrolysis reached its maximum value, the reaction was stopped by heating the solution at 90°C for 10 min, and the digest was cooled at room temperature and then centrifuged at 5,000 *g* for 15 min. The supernatant fraction containing FP hydrolysates was collected, freeze dried (104 h for a volume of 500 mL) then stored at 4°C until use. The respective final degrees of hydrolysis of the obtained hydrolysates were 19 and 9 per cent for Esperase-fenugreek proteins hydrolysate (EFPH) and Purafect-fenugreek proteins hydrolysate (PFPH). Protein contents of PFPH and EFPH were estimated at 90 and 92.5 per cent, respectively, and their amino acids composition was determined (Table I) according to Soufleros and Bertrand (1998).

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Table I. Amino acids composition of FPs and their hydrolysates

(%)	FP	EFPH	PFPH
Cys	0.7 ± 0.1	0.9 ± 0.0	0.8 ± 0.1
Met	0.8 ± 0.1	1.0 ± 0.0	1.0 ± 0.0
Asp/Asn	11.4 ± 0.1	12.6 ± 0.3	11.2 ± 0.0
Thr	3.6 ± 0.1	3.9 ± 0.1	4.7 ± 0.0
Ser	5.0 ± 0.1	3.9 ± 0.1	3.9 ± 0.1
Glu/Gln	12.7 ± 0.3	14.3 ± 0.2	14.0 ± 0.0
Pro	3.9 ± 0.1	3.2 ± 0.2	3.5 ± 0.0
Gly	3.5 ± 0.2	3.5 ± 0.1	3.8 ± 0.1
Ala	5.9 ± 0.1	5.9 ± 0.2	5.8 ± 0.1
Val	6.1 ± 0.1	5.0 ± 0.1	5.2 ± 0.1
Ile	6.5 ± 0.2	6.0 ± 0.0	5.7 ± 0.2
Leu	9.6 ± 0.1	10.0 ± 0.2	11.1 ± 0.1
Tyr	2.7 ± 0.3	2.9 ± 0.2	3.1 ± 0.0
Phe	3.5 ± 0.1	3.2 ± 0.0	3.4 ± 0.0
Lys	5.6 ± 0.3	5.8 ± 0.0	5.7 ± 0.2
His	3.1 ± 0.2	2.7 ± 0.1	2.5 ± 0.1
Trp	11.6 ± 0.2	11.1 ± 0.1	10.3 ± 0.0
Arg	4.2 ± 0.1	4.0 ± 0.2	3.9 ± 0.1

Notes: Amino acid composition was determined by HPLC (Soufleros and Bertrand, 1998). All measurements were performed in triplicate at the Regional Laboratory Quality Control and the Repression of fraud of Oran. Data are presented as mean ± SD

2.3 Animal treatment

In total, 30 male Wistar rats, weighing about 184 ± 7 g, purchased from Algiers Pasteur Institute (Algeria), were housed in cages in a room maintained at 22°C and 60 per cent relative humidity with a 12-h cycle of light and dark. Rats were given free access to tap water and food throughout the experiment. Animals were kept according to the general guidelines on the use of living animals in scientific investigation (Council of European Communities, 1986), and the institutional committee on animal care and use approved the protocol and use of rats (approval number DZ-TN371/13).

2.4 Experimental design

In all, 24 rats were fed during four weeks a hypercholesterolemic (HC) diet (Table II) containing 20 per cent casein supplemented with 1 per cent cholesterol and 0.5 per cent cholic acid and randomized into four groups of six rats each as follow:

- HC group receiving daily 1 g/kg body weight (BW) of casein.
- FP group daily receiving 1 g/kg BW of FPs.
- PFPH group daily receiving 1 g/kg BW of PFPH.
- EFPH group daily receiving 1 g/kg BW of EFPH.

A fifth group (control: **C**) was fed the same experimental diet without cholesterol/cholic acid and treated under the same conditions with 1 g of casein/kg BW given by gavage.

Animals were weighed twice a week, and food consumption was recorded daily. In the last week of the experiment, rats of each group were kept for three days in metabolic cages and feces were daily collected, then dried, weighed and stored at −40°C until analysis. At the end of the experiment, the overnight fasting rats were anesthetized with sodium

Ingredients	C	HC
Casein[b]	200	200
Corn starch[c]	597	582
Sucrose[d]	40	40
Sunflower oil[e]	50	50
Cellulose powder[f]	50	50
Mineral mix[g]	20	20
Vitamin mix[h]	40	40
Cholesterol[b]	0	10
Cholic acid[b]	0	5
Methionine[i]	3	3

Notes: [a]The diet contained 16 MJ/kg and was given in powdered form. [b]Sigma-Aldrich Chemie, Germany. [c]Maghnia Starch, Tlemcen, Algeria. [d]Commercial sugar, Cevital, Bejaia, Algeria. [e]Commercial oil (80% soy oil and 20% sunflower oil), Cevital, Bejaia, Algeria. [f]Agar, Liofilchem, Italie. [g]Mx SAFE 205B, Augy, France. [h]Vit SAFE 200, Augy, France. [i]Biochem Chemopharma, Canada

Table II. Composition of cholesterol-enriched diet (g/kg diet)[a]

pentobarbital (60 mg/kg). Blood samples were collected from the abdominal aorta and serum was obtained by low speed centrifugation (2000 *g*, 30 min at 4°C). Liver was excised, washed in ice-cold saline (150 mM NaCl) and stored at −40°C until analysis.

2.5 Biochemical analysis

Glycaemia was measured by using glucose oxydase kit and uric acid by uricase-peroxidase kit (Spinreact, Girona, Spain). Serum protein and albumin concentrations were measured by the methods of Biuret (Chronolab Kit, Barcelona, Spain) and Bromocresol Green (Biolabo kit, Maizy, France), respectively. Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activities were assayed using commercial kits (Spinreact, Girona, Spain).

Serum thiols were determined by a colorimetric assay as described by Sedlak and Lindsay (1968) using Ellman reagent. The results were calculated using the molar absorption coefficient of TNB $\varepsilon = 14.15 \times 10^3\ M^{-1}.cm^{-1}$ (Eyer *et al.*, 2003).

2.6 Hepatic and fecal lipid extraction

One gram of liver or feces was extracted with chloroform:methanol (2:1, v/v) according to the method of Folch *et al.* (1957). After solvent evaporation, lipids extract was dissolved in isopropyl alcohol and immediately analyzed.

2.7 Lipids analysis

Serum and liver triacylglycerol (TG) and total cholesterol (TC), unesterified cholesterol (UC) and phospholipids (PL) concentrations were measured by using commercial kits (Spinreact, Girona, Spain), (Biolabo, Maizy, France) and (Cypress, Langdorp, Belgium), respectively. Esterified cholesterol (EC) levels were deduced from the difference between TC and UC.

2.8 Lipoproteins fractionation and lipid sand apolipoproteins analysis

Fast protein liquid chromatography in Äkta-FPLC (Amersham Pharmacia Biotech, Barcelona, Spain) was used for analysis of the lipoproteins profile using 50 mM phosphate buffer sodium (PBS) as running buffer following the method described by Hubáček *et al.* (2008). A volume of 150 μL of each serum pool was loaded on a Superose[TM] 6 10/300 GL column (GE healthcare life sciences, Barcelona, Spain) and 0.5 mL of each fraction was

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collected. The elution was monitored at 280 nm for proteins content and collected in fractions.

The TC in each fraction was measured by a fluorometric method using Amplex Red (Molecular Probes, OR, USA). The phosphatidylcholine (PC) was determined by using the enzymatic procedure (Hojjati and Jiang, 2006) coupled to fluorometric detection (He *et al.*, 2002). Serum apolipoproteins (apo B, A-I and A-IV) were quantified by ELISA using specific polyclonal antibodies (Navarro *et al.*, 2005).

2.9 Lipoproteins oxidation susceptibility and high density lipoproteins antioxidant potential

The susceptibility of very low density lipoproteins and low density lipoproteins cholesterol (VLDL-LDL-c) to oxidation and the antioxidant potential of high density lipoproteins (HDL) to prevent low and very low density lipoproteins against oxidation in rats was measured according to the fluorometric method of Navab *et al.* (2001), as modified by Acín *et al.* (2006), using dichlorofluorescein (DCF) (Sigma, MO, USA) as an oxidation marker.

Two volumes of a preparation containing 100 μg/mL of VLDL-LDL-cholesterol and 4 μg/mL of HDL-cholesterol were mixed with 1/2 volume of 0.12 per cent sodium azide and 1/2 volume of DCF (80 μg/mL). After incubation for 3 h at 37°C, the intensity of the fluorescence of the DCF is measured. A control (for VLDL-LDL-c oxidation susceptibility) was prepared for each group by following the same steps with substituting HDL by PBS. The samples were analyzed in duplicate. The antioxidant index (AOI) of HDL is expressed as the percentage of non-HDL oxidation inhibition.

2.10 Paraoxonase activity assay

Paraoxonase activity (PON1) was determined in serum by measuring the conversion rate of phenyl acetate to phenol as described by Eckerson *et al.* (1983). In total, 5 μL of appropriately diluted serum was added to 200 μL of working reagent (20 mM Tris/HCl buffer, pH 8.0, containing 1 mM $CaCl_2$ and 4 mM phenyl acetate). The increase of the absorbance was recorded at 270 nm (25°C) every 30 s. One unit of arylesterase activity is equal to one μM of phenol formed per minute. The activity was expressed in U/mL based on the extinction coefficient of phenol (1,310 $M^{-1}cm^{-1}$). Blank sample containing buffer was used to correct for non-enzymatic hydrolysis.

2.11 Statistical analysis

Data are presented as mean ±SD and subjected to one way ANOVA and LSD-Fisher post hoc test. Differences are considered to be significant at $p \leq 0.05$ and were superscripted by (*) between HC rats and C group and by (#) between treated groups and HC one.

3. Results and discussion

3.1 Food intake, body weight and liver relative weight

The first objective of our study was to determine whether FP and their hydrolysates affect the dietary nutritional properties and hepatic function. Under our experimental conditions, neither food intake nor body weight changes were observed after the administration of FP, PFPH or EFPH, suggesting that FPs and their two hydrolysates, did not affect the nutritional efficiency of the given diet, permitting therefore a normal body weight growth. These results confirmed those previously published by Yahia *et al.* (2017) using chickpea protein hydrolysates. Similarly, Bettzieche *et al.* (2008) did not find any significant difference in body weight, after 20 days, between high cholesterol (HC)-fed rats receiving, daily, 1g of lupin or casein proteins, and those fed a control diet. Likewise, Yang *et al.* (2007) reported

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that the treatment with soy proteins and their hydrolysate (1g/day for 12 weeks) did not change body weight and food intake of rats.

However, when analysing liver relative weight, values were significantly increased by 1.3-fold in the four groups fed the hypercholesterolemic diet when compared to C group (Table III). This result could be explained by the high fat storage in liver of treated rats. Indeed, the higher lipid contents found in this organ indicated that FPs and their hydrolysates did not prevent liver lipid storage induced by the hypercholesterolemic diet.

3.2 Serum alanine aminotransferase and aspartate aminotransferase activities

In the present study, the hypercholesterolemic diet induced a slight increase of serum ASAT activity while ALAT activity was unchanged between the five groups. It has been previously reported by Ktari *et al.* (2015), that the hepatocytes were particularly vulnerable to lipid accumulation, which can cause deterioration of membrane integrity resulting in the release of several components, including transaminases, from their cytosol. The observed result suggested that liver damage was not advanced in HC rats (Kapravelou *et al.*, 2013) and could explain the unchanged ALAT activity in HC group. However, ASAT activity of the groups treated with FP and EPFH was 1.3-fold lower than value obtained with the HC group (p = 0.0003) and became comparable to that of C group **(**Table III**)**. This indicated that FP and EFPH protected liver against the deleterious effect of HC diet. These results were consistent with those of Kapravelou *et al.* (2013) who also observed that lupin protein hydrolysate attenuated hepatoxicity in high cholesterol-diet-fed rats.

3.3 Liver lipid contents

As depicted in Table IV, and compared to C group, all the liver lipids components were significantly higher in all HC-fed groups (HC, FP, PFPH and EFPH). The high contents of lipids, cholesterol and triglycerides in liver observed in HC non-treated animals vs C were probably a consequence of two mechanisms. Effectively, it has been estimated that, in rats, as much as 50 per cent of dietary fat enters the liver as remnant TG (Babin and Gibbons, 2009). The second mechanism is the stimulation of acyl-CoA cholesterol acyl transferase expression in liver by HC diet as mentioned by Wang *et al.* (2010). When compared to HC group, TC concentrations were reduced by 24 per cent in PFPH rats' liver (p = 0.03). UC levels in FP rats were increased by 27 per cent vs HC group (p = 0.07).

Groups	C	HC	FP	PFPH	EFPH
Initial BW *(g)*	185 ± 04	184 ± 09	184 ± 02	183 ± 04	189 ± 11
Final BW *(g)*	236 ± 18	240 ± 26	233 ± 13	249 ± 21	251 ± 30
Food intake *(g/d)*	17 ± 2	19 ± 4	22 ± 5	19 ± 5	20 ± 3
Food efficiency ratio *(%)*	0.13	0.12 ± 0.05	0.08 ± 0.01	0.12 ± 0.01	0.11 ± 0.03
Liver relative weight *(%)*	2.8 ± 0.1	3.7 ± 0.2*	3.8 ± 0.2*	3.7 ± 0.3*	3.8 ± 0.2*
ASAT *(U/l)*	58 ± 9	70 ± 7	47 ± 6#	72 ± 11	47 ± 7#
ALAT *(U/l)*	20 ± 3	23 ± 5	27 ± 7	26 ± 7	29 ± 8

Notes: C: Control group; HC: hypercholesterolemic group; FP: fenugreek protein group; PFPH: Purafect-fenugreek proteins hydrolysate group, EFPH: Esperase-fenugreek proteins hydrolysate group; BW: body weight; ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase; data are presented as mean of 6 rats per groups. Superscripted (#, *) means within a row indicate they are significantly different at $P < 0.05$, as following: *HC, FP, PFPH or EFPH vs C. #FP, PFPH or EFPH vs HC

Table III. Anthropometric parameters and serum transaminases activities of rats treated or not with FPs and their hydrolysates

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Liver triacylglycerol contents decreased (1.3-fold lower), but not significantly (p = 0.07), in rats fed FP. In PFPH and EFPH groups, these contents were significantly lower (−30 per cent, p = 0.02), both compared to HC. Furthermore, PL values did not show any significant difference between HC, FP, PFPH and EFPH rat groups. These results were in agreement with the works of Fassini *et al.* (2011) and Yang *et al.* (2007) who did not notice significant changes in liver cholesterol contents between HC and soy protein or its hydrolysate treated rats. In contrast, this work results were not wholly in line neither with the studies of Ferreira *et al.* (2015) and Kapravelou *et al.* (2013) who noticed a diminution of cholesterol and TG levels after protein or hydrolysate supplementation, nor with those of Bettzieche *et al.* (2008) who remarked that lupin protein had diminished only TG concentrations in liver vs HC group.

3.4 Glycaemia, serum lipids contents and fecal cholesterol excretion

Although glycaemia of all the experimental groups was normal, results showed a slight increase of blood glucose level in HC group compared with C group (p = 0.02). This was corrected after treatment with FP (p = 0.04 vs HC), suggesting a possible regulating effect of glycaemia by FP consumption.

The ability of FPs and their hydrolysates to prevent hypercholesterolemia complications in HC-diet-fed rat was further examined. After 28 days, and compared to control diet, HC diet induced a sharp increase of cholesterolemia by 439 per cent mainly in VLDL fraction. The TC increase, similarly observed in early works (Athmani *et al.*, 2015; Benomar *et al.*, 2015; Yahia *et al.*, 2017), was concomitant with hypertriglyceridemia state (1.6-fold higher) (Table V). These findings were in contrast with those reported by León-Espinosa *et al.* (2016) and Wang *et al.* (2010) showing that the HC diet decreased serum TG levels.

Compared to HC group, FP (p = 0.0003) and their hydrolysate produced by Esperase (p = 0.004) caused a significant decrease in serum TC (−53 and −34 per cent, respectively). The decreased cholesterolemia in FP group resulted from the low levels of the unesterified and esterified pools that were, respectively, reduced by 35 and 60 per cent, while, regarding EFPH group, only the esterified pool was decreased by −38 per cent, all compared with HC animals. The main hypothesis advanced to explain the observed cholesterol-lowering effect

Groups	C	HC	FP	PFPH	EFPH
mg/g liver					
Total lipids	71 ± 6	193 ± 21*	225 ± 18*	184±33*	198±25*
μmol/g liver					
TC	9.5 ± 0.7	67.0 ± 10.6*	75.0 ± 10.0*	50.9 ±6.7*#	76.2 ±9.3*
UC	2.7 ± 1.8	7.1 ± 1.7*	9.05 ± 1.9*#	7.3 ± 1.0*	5.8 ± 0.9*
CE	6.8 ± 1.3	59.9 ± 9.4*	64.4 ± 4.7*	43.6 ± 7.0*#	70.3 ± 10.7*
TG	6.5 ± 1.1	20.0 ± 2.8*	15.7 ± 5.0*	14.1 ± 2.1*#	14.7 ± 2.9*#
PL	34.0 ± 5.5	52.5 ± 6.2*	47.4 ± 8.3	64.5 ± 15.3*	51.8 ± 4.9*
CE/UC	2.51	8.43	7.11	5.97	12.12

Notes: C: Control group; HC: hypercholesterolemic group; FP: fenugreek protein group; PFPH: Purafect-fenugreek proteins hydrolysate group, EFPH: Esperase-fenugreek proteins hydrolysate group; TC: total cholesterol; UC: unesterified cholesterol; CE: cholesteryl ester; TG: triglycerides; PL: phospholipids; data are presented as mean ± SD of 6 rats per groups. Superscripted (#, *) means within a row indicate they are significantly different at $P < 0.05$, as following: *: HC, FP, PFPH or EFPH vs C. #FP, PFPH or EFPH vs HC

Table IV. Liver lipids contents in rats treated or not with FPs and their hydrolysates

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is the inhibition of cholesterol and lipids absorption (Ferreira *et al.*, 2015; Yahia *et al.*, 2017) as shown by the high fecal cholesterol excretion in FP-treated group ($P = 0.0001$). However, EFPH treated rats did not show any difference in fecal cholesterol compared with HC rats. In fact, Cho *et al.* (2007) reported that the *in vitro* effect of soy proteins on cholesterol micellar solubility was reduced after the hydrolysis of the intact proteins. It is possible that EFPH, via its small peptides fractions, passed the intestinal barrier and reached the systemic blood stream, then liver, where they could exert their bioactivities. This could also explain why EFPH (degree of hydrolysis =19 per cent), and not PFPH (degree of hydrolysis = 8 per cent), affected serum cholesterol concentration. Indeed, PFPH-treated rats exhibited 1.2-fold higher TC vs HC.

FP supply-induced serum TG decreased by 20 per cent when compared to HC group ($p = 0.04$). Moreover, the lowest PL levels were found with FP diet, unlike the others groups, where values were similar (Table V).

3.5 Cholesterol and phosphatidylcholine distribution on lipoproteins fractions

The effect of FP and their hydrolysates for the possible changes of cholesterol and PC distribution in different lipoproteins fractions was also assessed. With control diet, the obtained FPLC lipoproteins profile revealed that the highest cholesterol contents were found in HDL followed by VLDL. Nevertheless, this profile was changed by the HC diet as indicated by the highest amounts of VLDL followed by HDL (Figure 1). HDL-cholesterol (HDL-c) contents were markedly decreased in HC, FP and EFPH groups compared to C. However, compared with HC, FP and EFPH reduced VLDL-cholesterol (VLDL-c) and-phosphatidylcholine (VLDL-PC), while PFPH did not show any effect. Furthermore, in rats treated with PFPH, a redistribution of cholesterol toward HDL and cholesterol poor-HDL fractions was observed.

PC distribution in lipoproteins fractions of C and HC groups showed that PC was mainly observed in VLDL followed by HDL, smaller HDL and LDL. Compared to HC group, FP and EFPH induced a decrease in the phospholipid content of all lipoprotein fractions whereas PFPH exhibited the highest PC accumulation in the smaller HDL.

Table V. Glycaemia, serum lipids contents and fecal cholesterol excretion in treated or not with FPs and their hydrolysates

Groups	C	HC	FP	PFPH	EFPH
mmol/l					
Glycaemia	3.26 ± 0.22	4.99 ± 0.60*	3.75 ± 0.49#	4.87 ± 0.64*	3.78 ± 0.75
TC	1.43 ± 0.04	6.29 ± 1.14*	2.95 ± 0.46#	7.80 ± 1.79*	4.12 ± 0.76*#
UC	0.72 ± 0.01	1.62 ± 0.53*	1.06 ± 0.04#	1.58 ± 0.43*	1.23 ± 0.30*
CE	0.71 ± 0.05	4.67 ± 1.00*	1.88 ± 0.45#	6.21 ± 1.54*#	2.89 ± 1.04*#
TG	0.66 ± 0.30	1.09 ± 0.09*	0.87 ± 0.16#	1.14 ± 0.19	1.03 ± 0.06
PL	1.98 ± 0.42	2.27 ± 0.46	1.61 ± 0.10#	2.61 ± 0.54	1.86 ± 0.32
mg/day/rat					
Fecal cholesterol	1.8 ± 0.3	19.3 ± 5.8*	50.6 ± 6.9*#	17.6 ± 1.5*	21.6 ± 8.3*

Notes: C: Control group; HC: hypercholesterolemic group; FP: fenugreek protein group; PFPH: Purafect-fenugreek proteins hydrolysate group, EFPH: Esperase-fenugreek proteins hydrolysate group; TC: total cholesterol; UC: unesterified cholesterol; CE: cholesteryl ester; TG: triglycerides; PL: phospholipids; data are presented as mean ± SD of 6 rats per groups. Superscripted (#, *) means within a row indicate they are significantly different at $P < 0.05$, as following: *:HC, FP, PFPH or EFPH vs C. #FP, PFPH or EFPH vs HC

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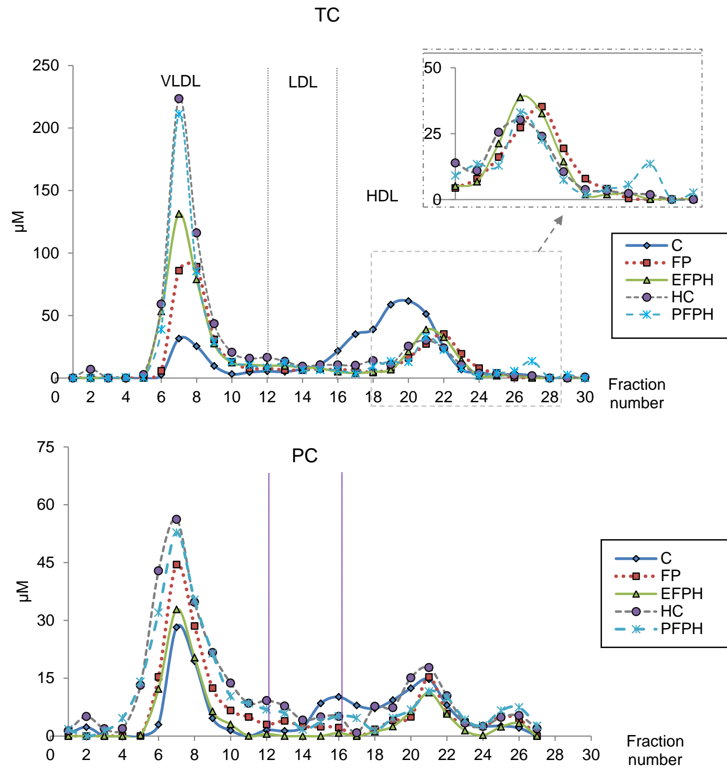

Notes: C: Control group; HC: hypercholesterolemic group; FP: fenugreek protein group; PFPH: Purafect-fenugreek proteins hydrolysate group, EFPH: Esperase-fenugreek proteins hydrolysate group; Serum lipoproteins were separated by FPLC and collected fractions analyzed for total cholesterol (TC), phosphatidylcholine (PC). Data are presented as representative profile of six pooled animals serum

Figure 1.
Cholesterol (TC) and phosphatidylcholine (PC) distribution in serum rats lipoproteins treated or not with FPs and their hydrolysates

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Similar results have been obtained by Ferreira *et al.* (2015) and Yang *et al.* (2007), showing that legume proteins and their hydrolysates decreased the non HDL-c amounts; however, they noticed an increase in HDL-c levels. The decreased HDL-c contents in all rats given the HC diet suggest that the reverse cholesterol transport was up regulated.

3.6 Serum apolipoproteins A-I and A-IV

Regarding C, HC, PFPH and EFPH groups, serum apolipoproteins B, A-I and A-IV were similar (Table VI). The HC and PL contents in VLDL, and the unchanged Apo B100, which reflect the number of VLDL and LDL particles, suggest that HC diet altered the composition of the VLDL core lipids rather than their synthesis or uptake by liver. Furthermore, Kang and Davis (2000) reported that when the amount of cholesterol increased in liver, the composition of the VLDL core lipids changed (i.e. increase the relative amount of cholesterol

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esters to triglycerides) while the number of VLDL particles, assembled and secreted by this organ, remains the same.

However, FP decreased Apo A-I (p = 0.03) and A-IV (p = 0.02) levels compared with C rats. In a recent work of Yahia *et al.* (2017), who studied the hypercholesterolemic effect of chickpea proteins hydrolysate on rats, no change in Apo A-I of HDL was founded, while an increase in Apo A-IV contents was noticed in this particle. Two possible mechanisms could explain the decrease of Apo A-I in FP group. The first one is the increase of its uptake by liver, and the second one consists on the decrease of its synthesis (Röhrl and Stangl, 2013). It is the first hypothesis rather than the second that could be advanced in the present study. Indeed, Apo A-I profile in FP rats serum showed a lower Apo A-I contents in their largest lipoproteins but not in the smallest ones. Further investigations should be undertaken to well understand the FP effect on HDL metabolism.

Apo A-IV is synthesized primarily by intestine and liver (Duka *et al.*, 2013). Therefore, the post-absorptive Apo A-IV level reflected indirectly its post-prandial metabolism. The lower Apo A-IV levels observed in FP serum rats vs C could be because of the reduced enterocyte lipid absorption by FP treatment which involved a down-regulation of Apo A-IV gene synthesis and then impaired its redistribution toward HDL (Duka *et al.*, 2013).

3.7 Proteins and oxidant stress markers

Compared with control diet, HC diet increased serum proteins. Nonetheless, after treatment with FP, PFPH or EFPH, the serum protein levels decreased (−15, 17 and 27 per cent, respectively compared to HC) and became similar to that of C group. Serum albumin did not show any significant difference among all groups (Table VII). However, and compared to animals fed control diet, serum uric acid markedly increased by HC feeding. This increase

Arbitary unit	C	HC	FP	PFPH	EFPH
Apo B	0.24 ± 0.04	0.24 ± 0.04	0.21 ± 0.01	0.24 ± 0.04	0.22 ± 0.03
Apo AI	0.64 ± 0.01	0.59 ± 0.06	0.53 ± 0.05*	0.57 ± 0.06	0.61 ± 0.05
Apo AVI	0.75 ± 0.04	0.69 ± 0.11	0.56 ± 0.06*	0.79 ± 0.06	0.75 ± 0.10

Notes: C: Control group; HC: hypercholesterolemic group; FP: fenugreek protein group; PFPH: Purafect-fenugreek proteins hydrolysate group, EFPH: Esperase-fenugreek proteins hydrolysate group; data are presented as mean ± SD of 6 rats per groups. Superscripted (*) means within a row indicate they are significantly different at $P < 0.05$, as following: *: HC, FP, PFPH or EFPH vs C

Table VI. Apolipoproteins B, A-I and A-IV contents in serums of rats treated or not with FPs and their hydrolysates

Groups	C	HC	FP	PFPH	EFPH
Proteins *(g/l)*	62 ± 15	73 ± 6	62 ± 1#	60 ± 4#	53 ± 6#
Albumin *(g/l)*	26 ± 4	28 ± 3	28 ± 3	27 ± 4	24 ± 3
Uric acid *(μM)*	125 ± 9	153 ± 8*	159 ± 10*	171 ± 16*#	158 ± 14*
Thiols (mM)	0.19 ± 0.07	0.49 ± 0.26	0.32 ± 0.13	0.44 ± 0.17	0.37 ± 0.12
Paraoxonase *(U/ml)*	109 ± 8	94 ± 8	94 ± 10	116 ± 6#	112 ± 8#

Notes: C: Control group; HC: hypercholesterolemic group; FP: fenugreek protein group; PFPH: Purafect-fenugreek proteins hydrolysate group, EFPH: Esperase-fenugreek proteins hydrolysate group; dara are presented as mean ± SD of 6 rats per groups. Superscripted (#, *) means within a row indicate they are significantly different at $P < 0.05$, as following: * HC, FP, PFPH or EFPH vs C. #FP, PFPH or EFPH vs HC

Table VII. Proteins and oxidant stress markers in rats treated or not with FPs and their hydrolysates

could probably be because of the higher xanthine oxidase activity mainly induced by the HC diet responsible of the hepatic injury. Similar hypothesis was previously proposed by Devrim *et al.* (2008). Indeed, uric acid is the end product of purine nucleotide catabolism by xanthine oxidase. It seems that neither FP nor their hydrolysates could prevent the increase of uric acid. In spite of the high thiol values observed in all treated groups, compared to C group, these differences were not significant.

3.8 Antioxidative action of HDL

Several studies have shown that exogenous antioxidants, especially originated from foods, are essential for counteracting oxidative stress (Shahidi *et al.*, 1992). In our protocol design, the potential of FPs and their hydrolysates to change oxidant/antioxidant status in hypercholesterolemic rat was assessed.

Products of lipid peroxidation, especially fatty acyl residues within phospholipids, present in oxidized-LDL can induce pro-inflammatory phenotypes in arterial wall cells, which contribute to endothelial dysfunction and apoptotic cell death, as the key steps of the initiation and progression of atherosclerosis lesions (Hafiane and Genest, 2013). Whereas, besides its role of ensuring the reverse transport of cholesterol, HDL plays an important function in protecting against non-HDL oxidation. This property is attributed to some proteins and enzymes (Hafiane and Genest, 2013). To evaluate the AOI of the treated rats HDL, non-HDL fraction of each group was incubated with and without the HDL of the same group and the inhibitory property of their oxidation was estimated.

In the absence of HDL, the highest VLDL-LDL oxidation level was observed in PFPH followed by EFPH groups, while the lowest oxidation was obtained in the C group (Table VIII). This could not be because of the amounts of lipids in these lipoproteins as, for the same levels of cholesterol, PL contents were almost the same. However, this high oxidability could be because of the nature of acyl groups in lipids. In FP group, the non-HDL fraction was less susceptible to oxidation than that of HC.

On the other hand, the HDL of PFPH and EFPH groups showed a remarkable antioxidative effect (−59 and −43 per cent, respectively) compared to HC group (−13 per cent) ($p = 0.001$). These results could be attributed to the elevated PON1 activity (20 and 15 per cent higher in PFPH and EFPH, respectively, compared to HC group; Table VII) probably because of the direct effect of some peptides within hydrolysates or to an up-regulation as response to the high oxidation of the non-HDL fraction. According to Aviram *et al.* (1999), PON1 is inhibited in presence of oxidized LDL. However, this inhibition is countred by antioxidants. Hence, it could be possible that the hydrolysates were implicated in the increase of PON1 activity.

Table VIII. AOI of HDL separated from serum of rats treated or not with FPs and their hydrolysates

Arbitary unit	C	HC	FP	PFPH	EFPH
(VLDL-LDL)	92.6 ± 0.0	166.0 ± 15.3*	89.5 ± 12.5$^{\#}$	257.8 ± 25.4*$^{\#}$	170.8 ± 34.7*
(VLDL-LDL) +HDL	76.4 ± 0.0	144.9 ± 6.4*	95.8 ± 12.2$^{\#}$	106.3 ± 19.2*$^{\#}$	97.5 ± 23.9*$^{\#}$

Notes: C: Control group; HC: hypercholesterolemic group; FP: fenugreek protein group; PFPH: Purafect-fenugreek proteins hydrolysate group, EFPH: Esperase-fenugreek proteins hydrolysate group; data are presented as mean ± SD of 6 rats per groups. Superscripted (#, *) means within a row indicate they are significantly different at $P < 0.05$, as following: *: HC, FP, PFPH or EFPH vs C. $^{\#}$FP, PFPH or EFPH vs HC

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4. Conclusion

FPs and EFPH exhibited an interesting hypocholesterolemic and hypotriglyceridemic activity, accompanied with an antioxidant property. The synergetic use of FPs and their Esperase hydrolysate could represent a promising nutraceutical ingredient in foods for the prevention against hypercholesterolemia complications. However, with the lack of information and investigations, it was not possible to settle if the hypocholesterolemic effect of FPs and their Esperase hydrolysate was mediated by their direct action on cellular or subcellular level or if it was just a consequence of the inhibition of cholesterol and lipid absorption in gut. Further works may bring more elucidations to well understand the exact mechanism by which they act.

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References

Acín, S., Navarro, M.A., Arbonés-Mainar, J.M., Guillén, N., Sarría, A.J., Carnicer, R., Surra, J.C., Orman, I., Segovia, J.C., De La Torre, R., Covas, M.I., Fernández-Bolaños, J., Ruiz-Gu, V. and Osada, J. (2006), "Hydroxytyrosol administration enhances atherosclerotic lesion development in apo E deficient mice", *The Journal of Biochemistry*, Vol. 140 No. 3, pp. 383-391.

Adler-Nissen, J. (1986), "A review of food hydrolysis specific areas", in Adler-Nissen, J. (Ed.), *Enzymic Hydrolysis of Food Proteins*, Elsevier, Copenhagen, pp. 57-109.

Athmani, N., Dehiba, F., Allaoui, A., Barkia, A., Bougatef, A., Lamri-Senhadji, M.Y., Nasri, M. and Boualga, A. (2015), "Sardina pilchardus and sardinella aurita protein hydrolysates reduce cholesterolemia and oxidative stress in rat fed high cholesterol diet", *Journal of Experimental and Integrative Medicine*, Vol. 5 No. 1, pp. 47-54.

Aviram, M., Rosenblat, M., Billecke, S., Erogul, J., Sorenson, R., Bisgaier, C.L., Newton, R.S. and La Du, B. (1999), "Human serum paraoxonase (PON1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants", *Free Radical Biology and Medicine*, Vol. 26 Nos 7/8, pp. 892-904.

Babin, P.J. and Gibbons, G.F. (2009), "The evolution of plasma cholesterol: direct utility or a 'spandrel' of hepatic lipid metabolism?", *Progress in Lipid Research*, Vol. 48 No. 2, pp. 73-91.

Belguith-Hadriche, O., Bouaziz, M., Jamoussia, K., Simmonds, M., El Feki, A. and Makni-Ayedi, F. (2013), "Comparative study on hypocholesterolemic and antioxidant activities of various extracts of fenugreek seeds", *Food Chemistry*, Vol. 138 Nos 2/3, pp. 1448-1453.

Benomar, S., Yahia, S., Dehiba, F., Guillen, N., Rodriguez-Yoldi, M.J., Osada, J. and Boualga, A. (2015), "Differential antioxidative and hypocholesterolemic responses to two fish protein hydrolysates (Sardina pilchardus and Boops boops) in cholesterol-fed rats", *Nutrition and Food Science*, Vol. 45 No. 3, pp. 448-466.

Bettzieche, A., Brandsch, C., Weiße, K., Hirche, F., Eder, K. and Stang, G.I. (2008), "Lupin protein influences the expression of hepatic genes involved in fatty acid synthesis and triacylglycerol hydrolysis of adult rats", *British Journal of Nutrition*, Vol. 99 No. 05, pp. 952-962.

Bouchenak, M. and Lamri-Senhadji, M. (2013), "Nutritional quality of legumes, and their role in cardiometabolic risk prevention: a review", *Journal of Medicinal Food*, Vol. 16 No. 3, pp. 185-198.

Boye, J.I., Aksay, S., Roufik, S., Ribéreau, S., Mondor, M., Farnworth, E. and Rajamohamed, S.H. (2010), "Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques", *Food Research International*, Vol. 43 No. 2, pp. 537-546.

Bukhari, S.B., Bhanger, M.I. and Memon, S. (2008), "Antioxidative activity of extracts from fenugreek seeds (Trigonella foenum-graecum)", *Pakistan Journal of Analytical and Environmental Chemistry*, Vol. 9, pp. 78-83.

Cho, S.J., Juillerat, M.A. and Lee, C.H. (2007), "Cholesterol lowering mechanism of soybean protein hydrolysate", *Journal of Agricultural and Food Chemistry*, Vol. 55 No. 26, pp. 10599-10604.

Christiane, K.F., Ellen, N. and Helene, L. (2009), "Allergenicity and antigenicity of fenugreek (Trigonella foenum-graecum) proteins in foods", *Journal of Allergy and Clinical Immunology*, Vol. 123 No. 1, pp. 187-194.

Cicero, A.F.G., Fogacci, F. and Colletti, A. (2017), "Potential role of bioactive peptides in prevention and treatment of chronic diseases: a narrative review", *British Journal of Pharmacology*, Vol. 174 No. 11, pp. 1378-1394.

Council of European Communities (1986), "Council instructions about the protection of living animals used in scientific investigations", *Official Journal of the European Communities (JO 86/609/CEE)*, Vol. L358, pp. 1-18. Brussels.

Devrim, E., Ergüder, I.B., Ozbek, H. and Durak, I. (2008), "High-cholesterol diet increases xanthine oxidase and decreases nitric oxide synthase activities in erythrocytes from rats", *Nutrition Research*, Vol. 28 No. 3, pp. 212-215.

Duka, A., Fotakis, P., Georgiadou, D., Kateifides, A., Tzavlaki, K., Von Eckardstein, L., Stratikos, E., Kardassis, D. and Zannis, V.I. (2013), "ApoA-IV promotes the biogenesis of apoA-IV-containing HDL particles with the participation of ABCA1 and LCAT", *Journal of Lipid Research*, Vol. 54 No. 1, pp. 107-115.

Eckerson, H.W., Wyte, C., La. and Du, B.N. (1983), "The human serum paraoxonase/arylesterase polymorphism", *American Journal of Human Genetics*, Vol. 35, pp. 1126-1138.

El Khoury, D. and Anderson, G.H. (2013), "Recent advances in dietary proteins and lipid metabolism", *Current Opinion in Lipidology*, Vol. 24 No. 3, pp. 207-213.

Elmnan, A., Balgees, A. and Mangara, J.L. (2012), "Effect of fenugreek (trigonella foenm greacum) seed dietary levels on lipid profile and body weight gain of rats", *Pakistan Journal of Nutrition*, Vol. 11 No. 11, pp. 1004-1008.

Eyer, P., Worek, F., Kiderlen, D., Sinko, G., Stuglin, A., Simeon-Rudolf, V. and Reiner, E. (2003), "Molar absorption coefficients for the reduced ellman reagent: reassessment", *Analytical Biochemistry*, Vol. 312 No. 2, pp. 224-227.

Fassini, P.G., Noda, R.W., Ferreira, E.S., Silva, M.A., Neves, V.A. and Demonte, A. (2011), "Soybean glycinin improves HDL-C and suppresses the effects of rosuvastatin on hypercholesterolemic rats", *Lipids in Health and Disease*, Vol. 10, pp. 165-171.

Ferreira, E.S., Amaral, A.L.S., Demonte, A., Zanelli, C.F., Capraro, J., Duranti, M. and Neves, V.A. (2015), "Hypocholesterolaemic effect of rat-administered oral doses of the isolated 7S globulins from cowpeas and adzuki beans", *Journal of Nutritional Science*, Vol. 4, pp. 1-9.

Ferrières, J., Bongard, V., Dallongeville, J., Arveiler, D., Cottel, D., Haas, B., Wagner, A., Amouyel, P. and Ruidavets, J.B. (2009), "Trends in plasma lipids, lipoproteins and dyslipidaemias in French adults, 1996-2007", *Archives of Cardiovascular Diseases*, Vol. 102 No. 4, pp. 293-301.

Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957), "A simple method for isolation and purification of total lipids from animal tissues", *Journal of Biology and Chemistry*, Vol. 226, pp. 497-509.

Garcia, M.C., Puchalska, P., Esteve, C. and Marina, M.L. (2013), "Vegetable foods: a cheap source of proteins and peptides with antihypertensive, antioxidant, and other less occurrence bioactivities", *Talanta*, Vol. 106, pp. 328-349.

Guallar-Castillón, P., Gil-Montero, M., León-Muñoz, L.M., Graciani, A., Bayán-Bravo, A., Taboada, J.M., Banegas, J.R. and Rodríguez-Artalejo, F. (2012), "Magnitud y manejo de la hipercolesterolemia en la población adulta de España, 2008-2010, el estudio ENRICA", *Revista Española de Cardiología*, Vol. 65 No. 6, pp. 551-558.

Habib-Martin, Z., Hammad, H., Afifi, F., Zihlif, M., Al-Ameer, H., Saleh, M., Abaza, I. and Nassar, Z. (2017), "In vitro and in vivo evaluation of the antiangiogenic activities of Trigonella foenum-graecum extracts", *Asian Pacific Journal of Tropical Biomedicine*, Vol. 7 No. 8, pp. 732-738.

Hafiane, A. and Genest, J. (2013), "HDL, atherosclerosis, and emerging therapies", Cholesterol, available at: http://dx.doi.org/10.1155/2013/891403

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He, X., Chen, F., Mcgovern, M.M. and Schuchman, E.H. (2002), "A fluorescence based, high-throughput sphingomyelin assay for the analysis of Niemann-Pick disease and other disorders of sphingomyelin metabolism", *Analytical Biochemistry*, Vol. 306 No. 1, pp. 115-123.

Hojjati, M.R. and Jiang, X.C. (2006), "Rapid, specific, and sensitive measurements of plasma sphingomyelin and phosphatidylcholine", *Journal of Lipid Research*, Vol. 47 No. 3, pp. 673-676.

Horwitz, W. (2000), *Official Methods of Analysis of AOAC International: Food Composition, Additives, Natural Contaminants, AOAC International*, Gaithersburg, MD.

Hubáček, J.A., Bobková, D., Bohuslavová, R. and Poledne, R. (2008), "Differences in expression of cholesterol 7α-hydroxylase between PHHC and wistar rats", *Folia Biologica*, Vol. 54 No. 1, pp. 18-23.

Institut National de Santé Publique (2007), "Enquête nationale santé: transition épidémiologique et système de santé. Projet TAHINA", *Ministère de la Santé, de la Population et de la Réforme Hospitaliaire*, El Biar.

Kang, S. and Davis, R.A. (2000), "Cholesterol and hepatic lipoprotein assembly and secretion", *Biochimica et Biophysica Acta*, Vol. 1529 Nos 1/3, pp. 223-230.

Kapravelou, G., Martínez, R., Andrade, A.M., Sánchez, C., Chaves, C.L., López-Jurado, M., Aranda, P., Cantarero, S., Arrebola, F., Fernández-Segura, E., Galisteo, M. and Porres, J.M. (2013), "Health promoting effects of lupin (lupinus albus var. multolupa) protein hydrolyzate and insoluble fiber in a diet-induced animal experimental model of hypercholesterolemia", *Food Research International*, Vol. 54 No. 2, pp. 1471-1481.

Khorshidian, N., Asli, M.Y., Arab, M., Mirzaie, A.A. and Mortazavian, A.M. (2016), "Fenugreek: potential applications as a functional food and nutraceutical", *Nutrition and Food Sciences Research*, Vol. 3 No. 1, pp. 5-16.

Ktari, N., Belguith-Hadriche, O., Ben Amara, I., Ben Hadj, A., Turki, M., Makni-Ayedi, F., Boudaouara, T., El Feki, A., Boualga, A., Ben Salah, R. and Nasri, M. (2015), "Cholesterol regulatory effects and antioxidant activities of protein hydrolysates from zebra blenny (Salaria basilisca) in cholesterol-fed rats", *Food and Function*, Vol. 6 No. 7, pp. 2273-2282.

Lemes, A.C., Sala, L., Ores, J.C., Braga, A.R.C., Egea, M.B. and Fernandes, K.F. (2016), "A review of the latest advances in encrypted bioactive peptides from protein-rich waste", *International Journal of Molecular Sciences*, Vol. 17 No. 6, pp. 950-973.

León-Espinosa, E.B., Sánchez-Chino, X., Garduño-Siciliano, L., Álvarez-González, R.I., Dávila-Ortiz, G., Madrigal-Bujaidar, E., Téllez-Medina, D.I. and Jiménez-Martínez, C. (2016), "Hypocholesterolemic and anticarcinogenic effect of vicia faba protein hydrolyzates", *Nutrition and Cancer*, Vol. 68 No. 5, pp. 856-864.

López-Barrios, L., Gutiérrez-Uribe, J.A. and Serna-Saldívar, S.O. (2014), "Bioactive peptides and hydrolysates from pulses and their potential use as functional ingredients", *Journal of Food Science*, Vol. 79 No. 3, pp. 273-283.

Madhava Naidu, M., Shyamala, B.N., Pura, N., J., Sulochanamma, G. and Srinivas, P. (2011), "Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds", *Food Science and Technology*, Vol. Vol. 44, pp. 451-456.

Meghwal, M. and Goswami, T.K. (2012), "A review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek", *Journal of Food Processing Technology*, Vol. 3, pp. 181-190.

Möller, N.P., Scholz-Ahrens, K.E., Roos, N. and Schrezenmeir, J. (2008), "Bioactive peptides and proteins from foods: indication for health effects", *European Journal of Nutrition*, Vol. 47 No. 4, pp. 171-182.

Mowla, A., Alauddin, M., Rahman, M.A. and Ahmed, K. (2009), "Antihyperglycemic effect of trigonella foenum-graecum (fenugreek) seed extract in alloxan-induced diabetic rats and its use in diabetes mellitus: a brief qualitative phytochemical and acute toxicity test on the extract", *African Journal of Traditional, Complementary and Alternative Medicines*, Vol. 6 No. 3, pp. 255-261.

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Nasri, M. (2017), “Protein hydrolysates and biopeptides: Production, biological activities, and applications in foods and health benefits: a review”, in Toldrá, F. (Ed.), *Advances in Food and Nutrition Research*, Academic Press, Cambridge, pp. 109-159.

Navab, M., Hama, S.Y., Hough, G.P., Subbanagounder, G., Reddy, S.T. and Fogelman, L.M. (2001), “A cell-free assay for detecting HDL that is dysfunctional in preventing the formation of or inactivating oxidized phospholipids”, *Journal of Lipid Research*, Vol. 42 No. 8, pp. 1308-1317.

Navarro, M.A., Carpintero, R., Acin, S., Arbones-Mainar, J.M., Calleja, L., Carnicer, R. and Surra, J.C. (2005), “Immune-regulation of the apolipoprotein A-I/C-III/A-IV gene cluster in experimental inflammation”, *Cytokine*, Vol. 31 No. 1, pp. 52-63.

Reddy, R.R.L. and Srinivasan, K. (2011), “Dietary fenugreek and onion attenuate cholesterol gallstone formation in lithogenic diet-fed mice”, *International Journal of Experimental Pathology*, Vol. 92 No. 5, pp. 308-319.

Röhrl, C. and Stangl, H. (2013), “HDL endocytosis and resecretion”, *Biochimica et Biophysica Acta*, Vol. 1831 No. 11, pp. 1626-1633.

Ruiz, J.C.R., Ancona, DB. and Campos, M.R.S. (2014), “Bioactive vegetable proteins and peptides in lipid-lowering; nutraceutical potential”, *Nutrición Hospitalaria*, Vol. 29, pp. 776-784.

Sarmadi, B.H. and Ismail, A. (2010), “Antioxidative peptides from food proteins: a review”, *Peptides*, Vol. 31 No. 10, pp. 1949-1956.

Sedlak, J. and Lindsay, R.H. (1968), “Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman’s reagent”, *Analytical Biochemistry*, Vol. 25, pp. 192-205.

Shahidi, F., Janitha, P.K. and Wanasundara, P.D. (1992), “Phenolic antioxidants”, *Critical Reviews in Food Science and Nutrition*, Vol. 32 No. 1, pp. 67-103.

Soufleros, E. and Bertrand, A. (1998), “Evaluation d’une méthode CLHP adaptée au dosage des acides aminés du vin”, *Vitis-Journal of Grapevine Research*, Vol. 37, pp. 43-53.

Srinivasan, K. (2013), “Dietary spices as beneficial modulators of lipid profile in conditions of metabolic disorders and diseases”, *Food & Function*, Vol. 4 No. 4, pp. 503-521.

Tóth, P.P., Potter, D. and Ming, E.E. (2012), “Prevalence of lipid abnormalities in the United States: the national health and nutrition examination survey 2003-2006”, *Journal of Clinical Lipidology*, Vol. 6 No. 4, pp. 325-330.

Udenigwe, C.C. and Howard, A. (2013), “Meat proteome as source of functional biopeptides”, *Food Research International*, Vol. 54 No. 1, pp. 1021-1032.

Udenigwe, C.C. and Rouvinen-Watt, K. (2015), “The role of food peptides in lipid metabolism during dyslipidemia and associated health conditions”, *International Journal of Molecular Sciences*, Vol. 16 No. 12, pp. 9303-9313.

Wang, Y.M., Zhang, B., Xue, Y., Li, Z.J., Wang, J.F., Xue, C.H. and Yanagita, T. (2010), “The mechanism of dietary cholesterol effects on lipids metabolism in rats”, *Lipids in Health and Disease*, Vol. 9, pp. 4-9.

Yahia, S., Benomar, S., Dehiba, F., Allaoui, A., Guillen, N., Rodriguez-Yoldi, M.J., Osada, J. and Boualga, A. (2017), “Hypocholesterolemic and antioxidant efficiency of chickpea (cicer arietinum) protein hydrolysates depend on its degree of hydrolysis in cholesterol-fed rat”, *Nutrition and Food Science*, Vol. 47 No. 2, pp. 254-269.

Yang, S.C., Liu, S.M., Yang, H.Y., Lin, Y.H. and Chen, J.R. (2007), “Soybean protein hydrolysate improves plasma and liver lipid profiles in rats fed high-cholesterol diet”, *The Journal of the American College of Nutrition*, Vol. 26 No. 5, pp. 416-423.

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