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Chemically induced common bean (*Phaseolus vulgaris* L.) sprouts ameliorate dyslipidemia by lipid intestinal absorption inhibition

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ABSTRACT

Common beans (*Phaseolus vulgaris* L.) are recommended as hypolipidemic adjuvants due to their high content of phytochemicals, which can be enhanced by germination and elicitation. The aim of this study was to evaluate the hypolipidemic effect of non-elicited [control (CS)] and chemically elicited [30 mM H_2O_2, 7 μM chitosan (CH), and 1 and 2 mM salicylic acid (SA)] bean sprouts. Rats were fed with a high fat and fructose diet (HFF) and supplemented with bean sprouts (10%) for twelve weeks. Control- and elicited-common bean sprouts significantly ($p < 0.05$) decreased serum triacylglycerides (TAG). Sprouts elicited with 1 and 2 mM SA increased fecal TAG excretion, which was related to the inhibition of pancreatic lipase enzyme activity. Hesperidin and soysaponin-I were identified as the main hypotriglyceridemic phytochemicals of bean sprouts according to the chemometric and *in silico* analyses. Therefore, the hypotriglyceridemic effect of SA-elicited bean sprouts was associated with decreased TAG intestinal absorption.

1. Introduction

Obesity is associated with dyslipidemia which is characterized by an abnormal metabolism of lipids, including high serum levels of triglycerides and total cholesterol and a low HDL serum levels (Klop, Elte, & Cabezas, 2013; Stone et al., 2013). The consumption of a hypercaloric diet plays an important factor in the onset and development of obesity-induced dyslipidemia. Accordingly, high fructose and fat diet (HFF) induces hyperlipidemia and lipid accumulation in adipose tissue (Tappy & Lê, 2010; Teodoro, Varela, Rolo, & Palmeira, 2014).

Regarding lifestyle interventions, the modification of dietary habits significantly improves dyslipidemia, which includes an increased uptake of bioactive compounds (Stone et al., 2013). Accordingly, common bean (*Phaseolus vulgaris* L) has been recommended to improve this metabolic alteration due to its high content of phytochemicals.

The hypolipidemic effect of common beans has been associated with the inhibition of intestinal lipid absorption (Kahlon, Smith, & Shao, 2005), regulation of appetite and satiety (Nilsson, Johansson, Ekström, & Björck, 2013; Spadafranca et al., 2013), inhibition of lipogenic enzymes and activation of lipid oxidation (Chávez-Santoscoy, Tovar, Serna-Saldivar, Torres, & Gutiérrez-Uribe, 2014; Kim, Hong, Jeon, & Kim, 2016). These beneficial effects have been related with their high content of dietary fiber, phenolic compounds, phytosterols, and saponins (Ramírez-Jiménez, Reynoso-Camacho, Tejero, León-Galván, & Loarca-Piña, 2015).

It has been reported that sprouting increases the content of several bioactive compounds in beans such as phenolic acids, flavonols, isoflavones, among others (Donangelo, Trugo, Trugo, & Eggum, 1995; Lin & Lai, 2006; López-Amorós, Hernández, & Estrella, 2006). In addition, chemically-induced elicitation has been reported to improve the phytochemical profile of several legumes (Gorelick & Bernstein, 2014; Świeca & Baraniak, 2014).

In a previous study we reported that elicitation of common beans during sprouting with salicylic acid (SA), chitosan (CH) and hydrogen peroxide (H_2O_2) increased their content of total polyphenols, flavonoids, and saponins, including *p*-coumaric acid, salicylic acid, gallic acid, caffeic acid, epigallocatechin, rutin, and quercetin. The main beneficial effect on the phytochemical profile was observed with SA treatment (Mendoza-Sánchez et al., 2016).

Several of these phytochemicals have been proved to reduce body adiposity and plasma lipids (Ramírez-Jiménez et al., 2015). Therefore, the increased content of these phytochemicals in chemically-induced common bean sprouts suggest an increased hypolipidemic potential. The aim of this study was to evaluate the effect of chemically-elicited

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bean sprouts on obesity and lipid metabolism in HFF-diet induced obese rats, and to identify the phytochemical compounds associated with the hypolipidemic effect of bean sprouts through a chemometric [partial least squares discriminant analysis (PLS-DA)] and *in silico* (molecular docking) approach.

2. Materials and methods

2.1. Materials

Dry common bean seeds (cultivar Dalia) were supplied by Campo Experimental Bajío (CEBAJ-INIFAP, Mexico). Dalia is a cultivar with high adaptation and yield stability, disease-resistant and tolerant to acidic soils (Acosta-Gallegos, Montero-Tavera, Jiménez-Hernández, Anaya-López, & Gonzalez-Chavira, 2014). Samples were manually cleaned up from impurities and soil contaminants.

2.2. Chemical-induced phytochemical elicitation

Non-elicited (control sprout, CS) and elicited Dalia bean sprouts were obtained as previously reported (Mendoza-Sánchez et al., 2016). Briefly, seeds were soaked in 1% sodium hypochlorite (1:6 w/v) for 30 min at room temperature. Then, seeds were drained, washed, and soaked in distilled water (1:6 w/v) for 6 h. Finally, hydrated seeds were placed in trays covered with wet filter paper. Trays were introduced into a germination chamber and filter paper was humidified daily with each elicitor dissolved in distilled water at the following concentrations: 7 μM chitosan (CH), 1 mM and 2 mM salicylic acid (SA), and 30 mM hydrogen peroxide (H_2O_2). Germinated seeds in distilled water were used as control. Germination was performed in darkness for 4 days at 25 °C. Then, germinated seeds (sprouts) were collected and immediately dried at 60 °C for 12 h, ground in a mill, and passed through a mesh with a particle size of 1 mm. Finally, flours were stored at 4 °C until analysis.

2.3. Quantitation of phytochemical compounds by HPLC-DAD-ESI-MS

The phytochemical profile was assessed by High Performance Liquid Chromatography (HPLC) (Agilent 1200) with a Diode Array Detector (DAD) coupled to a single-Quadrupole Mass Spectrometer (sQ-MS) using an atmospheric Electrospray Ionization (ESI) interphase (Agilent 1100) and a Zorbax Eclipse Plus (150 × 4.6 mm, 5 μm) column at 35 °C. The MS was operated in negative ionization mode using the following conditions: voltage capillary (4000 V), pressure nebulizer (40 psi), gas flow nebulizer (10 L/min), gas temperature nebulizer (300 °C), quadrupole voltage (150 V), fragmentor voltage (130 V). Mass spectra were acquired over a range of 80–1200 *m/z* (Pérez-Ramírez, González-Dávalos, Mora, Gallegos-Corona, & Reynoso-Camacho, 2017).

Phenolic compounds. Samples (0.25 mg of dried flours) were mixed with 0.5 mL of 50:50 (v/v) methanol:water (acidified with HCl to pH 2) and were sonicated for 30 s. Samples were centrifuged at 25,000*g* for 5 min at 4 °C. Supernatants were recovered, and then the extraction procedure was repeated with the residue with 0.5 mL of 70:30 (v/v) acetone:water. Finally, both supernatants were mixed, concentrated, and redissolved in 1 mL of methanol. Samples were filtered through a PTFE membrane (0.45 μm) and immediately injected in the chromatographic system. The separation was performed using as mobile phase (A) water:formic acid (99:1 v/v) and (B) acetonitrile under gradient conditions: 95/5 (A/B) from 0 to 20 min, 80/20 from 20 to 25 min, 60/40 from 25 to 30 min. Finally, the initial conditions were re-established and held for 5 min. The flow of the mobile phase was 0.8 mL/min. Absorbances were measured at 214, 256, 280, and 320 nm. Relative quantification was performed using a commercial standard for each class of phenolic compounds. Hydroxybenzoic acids were quantified with gallic acid, hydroxycinnamic acids with chlorogenic acids, flavanols with (+)-catechin, flavonols with rutin, flavanones with hesperidin, and isoflavones with genistein.

Phytosterols. Dried samples (50 mg) were extracted twice with 500 μL of n-hexane as previously described. The binary solvent system (flow rate of 0.8 mL/min) was performed using as mobile phase (A) methanol and (B) water:acetonitrile (99:1 v/v) under the following gradient: 85/15 (A/B) from 0 to 15 min, 100/0 (A/B) from 15 to 30 min. Finally, the initial conditions were re-established and held for 5 min. Absorbances were measured at 205 nm. Relative quantification was performed using β-sitosterol as standard.

Saponins. Dried samples (50 mg) were extracted twice with 500 μL of methanol:water 80:20 (v/v) as previously described. The binary system solvent (flow rate 0.4 mL/min) consisted of (A) acetonitrile:formic acid (99.9:0.1 v/v) and (B) water:formic acid (99.9:0.1 v/v) under the following gradient: 75/25 (A/B) from 0 to 3 min, 50/50 from 3 to 20 min, 20/80 from 20 to 30 min. Finally, the initial condition was re-established and held for 5 min. Absorbances were measured at 205 nm. Relative quantification was performed using soyasaponin I as standard.

2.4. In vivo experimental procedure

Fifty-six male Wistar rats (180 ± 10 g) were acquired from the Autonomous National University of México (UNAM, Mexico) and were maintained at 24 ± 1 °C under a 12/12 h light-dark cycle. The research protocol was reviewed and approved by the Bioethics Committee of the Autonomous University of Querétaro, Mexico. Animals were handled and cared as stated by the National Institutes of Health (NIH).

The rats were randomly divided into seven groups of eight rats each. The standard diet-fed group (SD) was fed with standard rodent diet [Lab Chow 5001; proteins 22%, lipids 6.4% (1% as saturated fat), and carbohydrates 48%]. The control and treated-obese groups were fed with a high fat (20% lard)/high fructose (18%) diet [proteins 15%, lipids 24% (20% as saturated fat) and carbohydrates 48%)] alone (HFFD) or supplemented with 10% control sprout (CS) and chemically-elicited bean sprouts with CH (7 μM), H_2O_2 (30 mM) or SA at 1 mM (SA-1) or 2 mM (SA-2) [proteins 17%, lipids 27% (20% as saturated fat), and carbohydrates 51%]. All diets were prepared weekly and stored at −20 °C until use. Water and food were provided *ad libitum* during all the experimental period.

Body weight and food intake were recorded daily. After twelve weeks, animals were placed in metabolic cages for feces collection, which were kept at −80 °C until analysis. Then, animals were fasted overnight and anesthetised. Blood was collected by cardiac puncture and rats were further euthanized. Visceral adipose tissue was recollected for further analysis.

2.5. Histological analysis of adipose tissue

A portion of visceral adipose tissue was stored in 10% neutral buffered formalin (pH 7.4) and processed to obtain 4 μm-thick tissue sections stained with Hematoxylin and Eosin (H&E). Adipocytes were observed at 200×, photographed, and their mean diameter was estimated by measuring 50 adipocytes from 3 randomly selected fields per sample. Results were expressed as μm.

2.6. Determination of biochemical parameters

Total cholesterol (TC), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglycerides (TAG) serum levels were determined with enzymatic-colorimetric kits (Spinreact, Santa Coloma, Spain).

2.7. Triglyceride content in feces

Feces (50 mg) were homogenized with 100 μL of sodium chloride and 400 μL of chloroform:methanol (2:1). Samples were centrifuged at

7000*g* for 10 min and the supernatant was incubated at −20 °C for 2 h, centrifuged at 11,200*g* for 5 min at 4 °C, and the lower phase was recovered and evaporated to dryness. Finally, the fecal TAG content was quantified with an enzymatic-colorimetric kit (Spinreact, Santa Coloma, Spain).

2.8. In vitro lipase inhibition assay

The *in vitro* pancreatic lipase inhibition assay was performed as reported by McDougall, Kulkarni, and Stewart (2009) with minor modifications (Pérez-Ramírez et al., 2017).

2.9. In silico studies

Molecular docking was performed with *Rattus norvegicus* lipase [SWISS-PROT: Pancreatic lipase-related protein (LIPR2_RAT P54318)] and the phytochemicals associated with *in vitro* pancreatic lipase inhibition according to the multivariate analysis. The ligands evaluated in this study were (PubChem CID): protocatechuic acid (72), 4-hydroxybenzoic acid (135), rutin (*5280805*), hesperidin (*10621*), soyasaponin I (*12209*), and campesteryl 3-β-glucopyranoside (*70699334*). Ligand structures were first drawn (3D) in ChemSketch v.11.02 and converted to a docking format (.pdb). The adjustment of angles/torsion of each ligand was carried out in the Avogadro v2.0 software (Hanwell et al., 2012), and its molecular structure with the enzyme (as rigid template) was explored using UCSF chimera software v1.11. The ligand optimization (preferred molecular orientation) with the least binding energy (ΔG_{bind}) was recorded for each ligand. Molecular interactions of two most plausible inhibitors were analyzed by considering the distances between atoms (Å), as well as their orientations and electronegativity.

Molecular properties and drug-likeness of each ligand (SMILES sequence) were evaluated by chemoinformatics. Molecular properties [Octanol-water partition coefficient (MiLogP), topological polar surface area (TPSA, Å), molecular weight (MW, g/mol), hydrogen bond donor (HBDC, #)/acceptor (HBAC, #) count, rotatable bond count (RBC, H) and number of Lipinski's "rule of five" violations (LPV, #)] and bioactivity scores [G-protein-coupled receptor ligand (1), ion channel modulator (2), kinase inhibitor (3), nuclear receptor ligand (4), protease (5) & enzyme (6) inhibitor] were calculated using the ©Molinspiration WEB tool kit (http://www.molinspiration.com).

2.10. Statistical analysis

The phytochemical profile results are expressed as mean ± standard deviation, whereas the *in vitro* and *in vivo* results are expressed as mean ± standard error. Data were analyzed by one-way analysis of variance (ANOVA) and differences among treatments were determined by comparison of means using the Tukey's test ($p < 0.05$). Partial Least Squares Discriminant Analysis (PLS-DA) was performed with centered and scaled data to obtain the model coefficients that associate the phytochemicals of common bean sprouts with the *in vivo* anti-obesogenic and hypolipidemic effects, as well as the *in vitro* pancreatic lipase inhibitory activity. All analyses were carried out using the JMP software v13.0.

3. Results and discussion

3.1. Effect of control- and elicited-common bean sprouts on body weight and adipocyte hypertrophy in obese rats

The effect of control and elicited-common bean sprouts on obesity and lipid profile in HFF diet-fed rats is shown in Fig. 1. After twelve weeks of the administration of an obesogenic diet, the HFFD group showed increased body weight as compared with the SD group (12.8%). Accordingly, it has been reported that prolonged feeding with fat-enriched diets induces a rapid body weight gain in rats (10–20%) as compared to SD-fed rats (Buettner, Schölmerich, & Bollheimer, 2007).

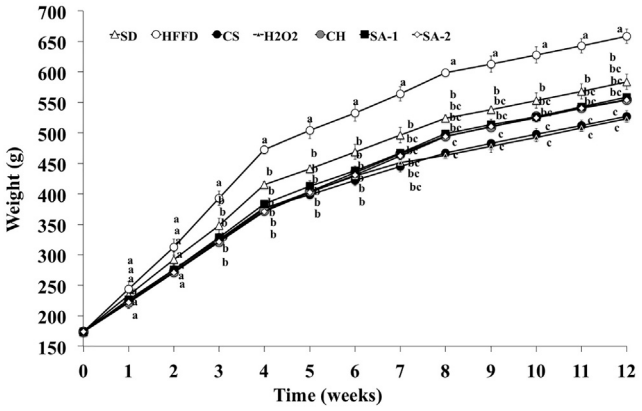

Fig. 1. Body weight of rats fed a HFFD and supplemented with control- chemically-elicited common bean sprouts. Data are presented as mean values (n = 8) and error bars represent standard error. Different letters indicate significant statistical difference by Tukeýs test ($p < 0.05$). Diets: standard (SD), high fat/fructose (HFFD), and control (CS)-, 30 mM hydrogen peroxide (H_2O_2)-7 μM chitosan (CH)-, salicylic acid 1 mM (SA-1)- and 2 mM (SA-2)-elicited common bean sprouts.

Rats supplemented with SA- and CH-elicited sprouts showed significantly decreased body weight as compared with HFF diet-fed rats (~15%), showing similar values than the SD group. Rats supplemented with CS and H_2O_2-elicited sprouts showed the lowest body weight throughout the experiment with values slightly lower than those from the SD group. Nevertheless, no statistical differences were observed.

HFF diet is known to cause many metabolic alterations, even worse than those observed with high fat diets (Tappy & Lê, 2010; Teodoro et al., 2014). Weight gain of animals fed HFFD is often associated to a higher TAG deposition in adipose tissue (Buettner et al., 2007). Accordingly, the HFFD group showed increased adipocyte hypertrophy as observed in increased adipocyte mean diameter as compared to the SD group (1.5 fold) (Fig. 2). The supplementation with all common bean sprouts significantly ($p < 0.05$) decreased adipocyte hypertrophy, showing similar values than the SD group. The anti-obesogenic effect of common bean sprouts was not associated with a decreased food intake, since all HFFD-fed rats showed a similar food intake throughout the experiment (15–18 g per day).

Common bean sprouts phytochemical profile was assessed by HPLC-DAD-MS followed by a PLS-DA analysis to identify the bioactive compounds associated with their anti-obesogenic effect. The phytochemical profile of the control- and elicited-common bean sprouts is shown in Table 1.

Nine phenolic acids, eight flavonoids, 4 saponins, and 6 phytosterols were identified and quantified in common bean sprouts. Interestingly, dicaffeoylquinic acid, chlorogenic acid (-caffeoylquinic acid) and p-coumaric acids were identified as the major phenolic acids of common bean sprouts, and genistein, daidzein, and rutin were identified as the major flavonoids, which were significantly ($p < 0.05$) increased with all chemical elicitors.

Regarding saponins, soysaponin I was identified as the major saponin in common bean sprouts, which was significantly ($p < 0.05$) increased only with 2 mM SA treatment. Δ7-Avenasterol, campesteryl 3-β-D-glucopyranoside, and stigmasterol 3-β-D-glucopyranoside were identified as the major phytosterols of common bean sprouts. Δ7-Avenasterol was significantly ($p < 0.05$) increased only with 1 mM SA treatment, campesterol 3-β-D-glucopyranoside was significantly ($p < 0.05$) increased with 1 and 2 mM SA and CH treatments, whereas stigmasterol 3-β-D-glucopyranoside was increased with all chemical elicitors.

Interestingly, the PLS-DA model coefficients indicated that β-

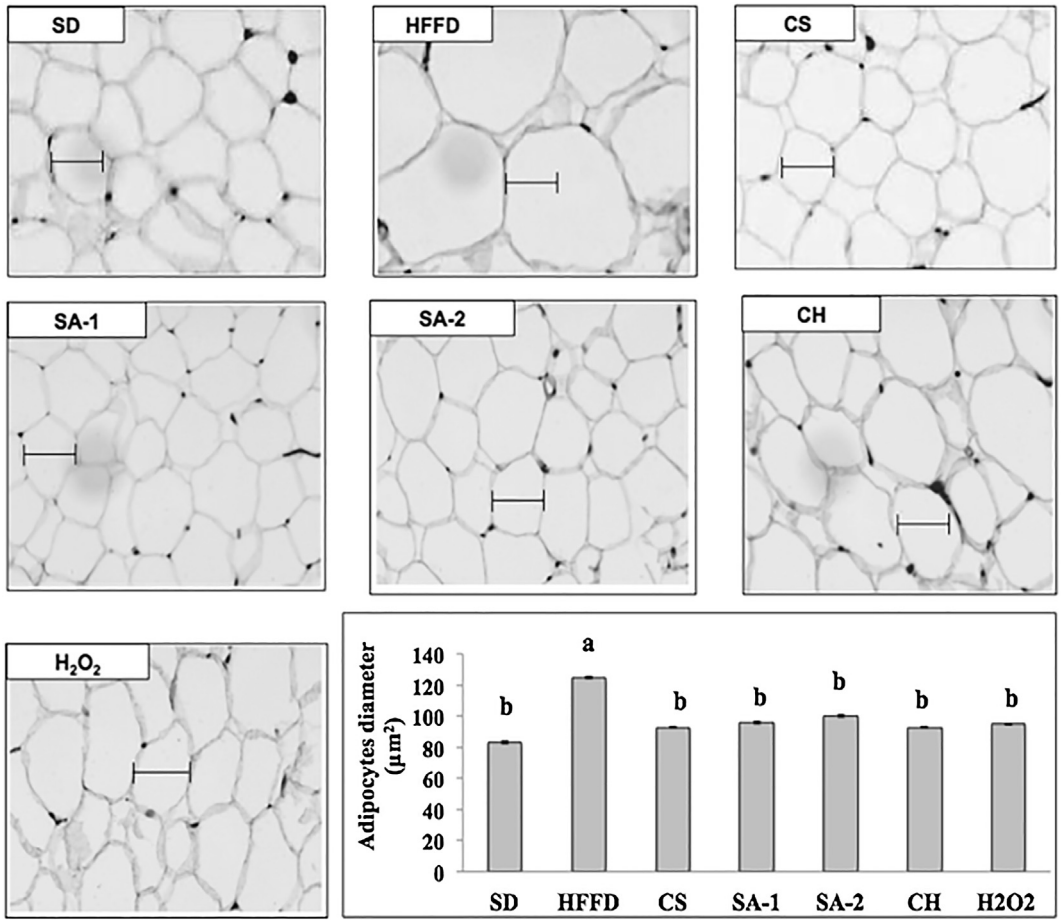

Fig. 2. Adipose tissue histology analysis and adipocyte diameter of rats feed with diets: standard (SD), high fat/fructose (HFFD), and HFFD supplemented with control (CS)-, 30 mM hydrogen peroxide (H_2O_2)- 7 μM chitosan (CH)-, 1 mM salicylic acid (SA-1)- and 2 mM salicylic acid (SA-2)-elicited common bean sprouts. At 200x magnification.

sitosterol and β-campesterol are associated with the anti-obesogenic effect of common bean sprouts (Table 3). Accordingly, it has been reported that bean phytosterols are fermented in colon and their fermentation products decrease hepatic lipogenesis and increase β-oxidation, which have been related to body weight reduction in obese animals (Chavez-Santoscoy et al., 2013).

3.2. Effect of control- and elicited-common bean sprouts on the lipid profile of obese rats

The effect of control- and elicited-common bean sprouts on dyslipidemia in obese rats is showed in Table 2. Rats fed with HFFD developed dyslipidemia which was characterized by increased serum TC, LDL, and TAG levels (20, 147, and 155%, respectively) and decreased serum HDL levels (34%) as compared to SD-fed rats. The supplementation with control- and elicited-common bean sprouts significantly ($p < 0.05$) decreased serum TC levels as compared with HFFD-fed rats (14–20%), but no significant ($p < 0.05$) differences were observed between treatments.

In addition, the supplementation with CS and 1 mM SA elicited-common bean sprouts significantly ($p < 0.05$) reduced LDL levels as compared to the HFFD group (28 and 24%, respectively), whereas the supplementation with 1 and 2 mM SA and CH elicited-common bean sprouts significantly ($p < 0.05$) increased HDL serum levels as compared to the HFFD group (16, 19, and 24%, respectively).

The greatest beneficial effect of elicited-common bean sprouts was on TAG serum levels. All common bean sprouts significantly ($p < 0.05$) decreased serum TAG levels; nevertheless, most elicited-common bean sprouts exerted the greatest hypotriglyceridemic effect. Control-common bean sprout supplementation decreased serum TAG levels by 39% as compared with the HFFD group, whereas chemically elicited-common bean sprouts decreased serum TAG levels by 22–54%. The greatest beneficial effect was observed with bean sprouts elicited with 7 μM CH and 1 and 2 mM SA treatments, whereas the lowest hypotriglyceridemic effect was observed with bean sprouts elicited with 30 mM H_2O_2. Therefore, these results suggest that elicitation with CH and SA enhances the hypotriglyceridemic effect of common bean sprouts, but not their anti-obesogenic and hypocholesterolemic effects.

According to PLS-DA model coefficients, the effect of common bean sprouts on lowering TC and LDL serum levels were associated with Δ7-avenasterol (−0.1152 and −0.1356, respectively) (Table 3). Accordingly, control- and 1 mM SA elicited-common bean sprouts showed the highest content Δ7-avenasterol and rats supplemented with these sprouts showed the lowest TC and LDL serum levels. Conversely, caffeic acid, genistein, and kaempferol were associated with increased TC serum levels. Moreover, caffeic acid and genistein were also associated with increased LDL serum levels.

On the other hand, hesperidin, soysaponin I, soysaponin βg, and soysaponin αg were associated to the hypotriglyceridemic effect of common bean sprouts (−0.1144, −0.1067, −0.1118, and −0.1271, respectively) (Table 3). Accordingly, these phytochemicals were increased with the application of 1 and 2 mM SA and 7 μM CH as compared to the control common bean sprout, and rats supplemented with these chemically elicited-common bean sprouts showed the lowest serum TAG levels.

It has been reported that polyphenols and saponins are potent

Table 1
Phytochemical profile of control- and elicited-common bean (*Phaseolus vulgaris* L.) sprouts by HPLC-DAD-ESI-MS.

Phytochemical compound	Rt (min)	(M-H)	Control sprout	30 mM H_2O_2	7 μM CH	1 Mm SA	2 mM SA
Phenolic acids							
Gallic acid*	2.4	169	8.8 ± 0.3^{c}	19.7 ± 0.4^{a}	4.8 ± 0.3^{d}	18.8 ± 0.1^{b}	18.1 ± 0.1^{b}
4-Hydroxybenzoic acid*	3.4	137	19.0 ± 0.2^{e}	26.2 ± 0.3^{c}	25.1 ± 0.1^{d}	34.8 ± 0.2^{b}	35.7 ± 0.1^{a}
Chlorogenic acid*	9.9	353	24.4 ± 0.2^{d}	35.3 ± 1.0^{b}	31.3 ± 0.4^{c}	33.9 ± 0.2^{bc}	82.7 ± 1.2^{a}
Caffeic acid*	11.3	179	6.3 ± 0.1^{e}	23.9 ± 0.6^{c}	25.4 ± 0.1^{b}	14.7 ± 0.1^{d}	28.9 ± 0.2^{a}
Ferulic acid*	12.4	193	2.7 ± 0.2^{e}	7.5 ± 0.3^{a}	5.6 ± 0.1^{b}	4.8 ± 0.1^{c}	4.1 ± 0.0^{d}
p-Coumaric acid*	13.2	163	49.9 ± 0.1^{e}	85.2 ± 1.2^{c}	69.4 ± 0.5^{d}	94.8 ± 0.0^{a}	90.0 ± 0.2^{b}
Dicaffeoylquinic acid*	13.5	515	83.9 ± 2.5^{d}	169.0 ± 1.2^{b}	159.1 ± 2.0^{c}	159.2 ± 0.2^{c}	252.2 ± 2.4^{a}
Sinapic acid*	13.7	223	5.9 ± 0.1^{a}	5.7 ± 0.2^{ab}	5.6 ± 0.1^{ab}	5.6 ± 0.1^{ab}	5.4 ± 0.1^{b}
Protocatechuic acid*	14.9	153	11.0 ± 0.2^{b}	LDL	LDL	16.0 ± 0.0^{a}	16.4 ± 0.2^{a}
Flavonoids							
Epicatechin*	7.8	189	7.7 ± 0.0^{d}	9.7 ± 0.1^{b}	10.3 ± 0.1^{a}	9.8 ± 0.1^{b}	8.4 ± 0.2^{c}
Catechin*	10.1	189	7.2 ± 0.1^{c}	11.4 ± 0.5^{a}	7.7 ± 0.1^{c}	10.3 ± 0.1^{b}	10.3 ± 0.1^{b}
Rutin*	11.7	609	55.6 ± 0.8^{d}	60.7 ± 0.3^{c}	60.5 ± 0.2^{c}	87.3 ± 0.1^{b}	115.0 ± 1.4^{a}
Hesperidin*	13.5	609	10.6 ± 0.1^{d}	10.3 ± 0.2^{d}	14.6 ± 0.2^{c}	22.2 ± 0.2^{a}	19.2 ± 0.1^{b}
Daidzein*	15.4	253	37.9 ± 0.2^{e}	59.5 ± 1.8^{d}	68.8 ± 1.2^{c}	78.4 ± 0.7^{b}	97.3 ± 0.3^{a}
Quercetin*	15.9	301	12.4 ± 1.0^{d}	24.8 ± 1.8^{a}	23.1 ± 1.7^{a}	18.9 ± 1.3^{b}	15.7 ± 1.3^{c}
Kaempferol*	16.3	285	LDL	2.1 ± 0.1^{b}	5.1 ± 0.4^{a}	1.8 ± 0.1^{b}	4.5 ± 0.3^{a}
Genistein*	17.3	269	60.0 ± 1.7^{d}	138.3 ± 1.0^{b}	136.3 ± 0.8^{b}	119.7 ± 0.7^{c}	164.5 ± 0.6^{a}
Saponins							
Phaseoside I	9.9	1252	5.0 ± 0.1^{c}	5.8 ± 0.1^{e}	4.5 ± 0.2^{d}	5.5 ± 0.0^{b}	6.7 ± 0.0^{a}
Soysaponin Bb (I)*	11.7	942	19.2 ± 0.3^{b}	12.9 ± 0.8^{d}	16.2 ± 0.1^{c}	17.1 ± 0.2^{c}	21.0 ± 0.1^{a}
Soysaponin βg	26.6	1068	9.4 ± 0.1^{c}	8.0 ± 0.0^{e}	11.9 ± 0.1^{a}	10.6 ± 0.1^{b}	8.5 ± 0.1^{d}
Soysaponin αg	27.3	1074	6.2 ± 0.1^{c}	5.6 ± 0.1^{d}	8.2 ± 0.1^{a}	7.7 ± 0.1^{b}	6.4 ± 0.0^{c}
Phytosterols							
Fucosterol	2.7	411	7.5 ± 0.1^{c}	6.6 ± 0.1^{c}	35.0 ± 0.7^{a}	7.6 ± 0.4^{c}	10.8 ± 0.4^{b}
β-Sitosterol*	7.3	413	19.4 ± 0.2^{c}	39.7 ± 1.2^{a}	20.3 ± 0.6^{bc}	21.2 ± 0.4^{bc}	22.1 ± 0.2^{b}
β-Campesterol	8.7	399	9.8 ± 0.2^{b}	15.3 ± 0.6^{a}	7.0 ± 0.2^{c}	8.4 ± 0.2^{b}	9.3 ± 0.5^{b}
Δ7-Avenasterol	16.9	411	72.3 ± 0.4^{b}	69.5 ± 1.1^{b}	71.0 ± 2.2^{b}	79.9 ± 0.4^{a}	68.4 1.0^{b}
Campesteryl 3-β-glucopyranoside	20.4	561	66.6 ± 1.6^{c}	68.7 ± 1.1^{c}	58.6 ± 1.2^{d}	95.5 ± 1.4^{a}	74.5 ± 0.4^{b}
Stigmasteryl 3-β-glucopyranoside	20.7	573	10.7 ± 0.1^{c}	23.5 ± 0.9^{b}	26.5 ± 0.3^{a}	27.9 ± 0.6^{a}	28.1 ± 0.8^{a}

Values are reported as mean ± standard deviation (n = 3). Values are expressed as μg/g. Different letters in the same row indicate significant ($P < 0.05$) differences. LDL, lower than detection limit. *Compounds identified by comparison with commercial standards.

inhibitors of pancreatic lipase activity (Buchholz & Melzig, 2015; Teodoro et al., 2014; Singh, Suresh, Bayineni, & Kadeppagari, 2015) and interfere with TAG intestinal absorption (Jakobek, 2015; Tippel, Gies, Harbaum-Piayda, Steffen-Heins, & Drusch, 2017). Therefore, the hypotriglyceridemic effect of common bean sprouts could be related to the inhibition of TAG intestinal digestion or absorption. To test this hypothesis, we carried out the quantification of fecal TAG in the rodent model and the determination of *in vitro* lipase inhibitory activity assay.

The effect of control- and elicited-common bean sprouts on fecal TAG levels is shown in Table 2. The administration of the HFFD increased the fecal TAG excretion by 50% as compared with the SD, which is related to the high fat content of the obesogenic diet. Interestingly, the supplementation with 1 and 2 mM elicited-common bean sprout significantly ($p < 0.05$) increased fecal TAG content as compared to the HFFD group (26 and 31%, respectively), whereas no significant ($p < 0.05$) effect was observed with the control- and the H_2O_2 and CH elicited-common bean sprouts. Therefore, these results suggest that the hypotriglyceridemic effect of SA elicited-common bean sprouts is associated with decreased TAG intestinal absorption.

The hypotriglyceridemic effect of common bean sprouts can also be associated with decreased TAG intestinal digestion. Therefore, we evaluated their effect on pancreatic lipase inhibitory activity using an *in vitro* assay (Table 2). All chemically elicited-common bean sprouts showed a higher inhibitory activity against pancreatic lipase as compared to the control-common bean sprout (20–118%). Interestingly, the greatest inhibitory activity was shown by 1 and 2 mM SA elicited-common bean sprouts. Therefore, these sprouts inhibit TAG intestinal digestion and absorption, leading to decreased serum TAG levels.

The PLS-DA model coefficients indicated that the inhibitory activity of common bean sprouts against pancreatic lipase is associated with their content of 4-hydroxybenzoic acid (0.1035), protocatechuic acid (0.1007), hesperidin (0.1110), rutin (0.1070), soysaponin I (0.1039),

Table 2
Physiological outputs of rats fed a high fat and fructose diet and supplemented with control- and elicited-common bean (*Phaseolus vulgaris* L.) sprouts.

Groups	TC (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	TAG (mg/dL)	Fecal TAG (mg/g)	Lipase inhibition (%)
SD	99 ± 8^{b}	64 ± 13^{c}	64 ± 10^{a}	83 ± 14^{d}	30 ± 4^{c}	–
HFFD	119 ± 3^{a}	158 ± 29^{a}	42 ± 6^{b}	212 ± 34^{a}	45 ± 2^{b}	–
CS	99 ± 6^{b}	114 ± 9^{b}	46 ± 6^{bc}	130 ± 18^{bc}	47 ± 4^{b}	12.7 ± 0.7^{c}
H_2O_2	102 ± 10^{b}	143 ± 18^{ab}	49 ± 3^{bc}	165 ± 14^{b}	48 ± 4^{b}	15.5 ± 0.7^{b}
CH	102 ± 15^{b}	134 ± 15^{ab}	49 ± 4^{c}	104 ± 11^{cd}	47 ± 3^{b}	15.2 ± 1.3^{b}
SA-1	99 ± 7^{b}	120 ± 21^{b}	50 ± 7^{c}	97 ± 17^{cd}	61 ± 3^{a}	25.9 ± 0.7^{a}
SA-2	104 ± 9^{b}	140 ± 17^{ab}	52 ± 4^{c}	106 ± 10^{cd}	59 ± 2^{a}	27.8 ± 0.8^{a}

Data is expressed as average ± standard error (n = 8). Different letters in a same column indicate statistical differences by Tukeý́s test ($p < 0.05$). Diets: standard (SD), high fat/fructose (HFFD), and HFFD supplemented with non (CS)-, 30 mM hydrogen peroxide (H_2O_2)- 7 μM chitosan (CH)-, 1 mM salicylic acid (SA-1)- and 2 mM salicylic acid (SA-2)-elicited Dalia bean sprout. Triacylglycerides (TAG), total cholesterol (TC) low-density lipoprotein (LDL), and high-density lipoprotein (HDL). +At 200x magnification.

Table 3
Predicted bioactivity of the phytochemicals identified in common bean sprouts by partial least squares discriminant analysis (PLS-DA).

Family	Compound	Model coefficients[a]				
		BW	TC	LDL	TAG	LIP
PC	Dicaffeoylquinic acid	0.0598	0.0900	0.0883	−0.0195	0.0569
PC	Chlorogenic acid	0.0586	0.0715	0.0673	−0.0268	0.0580
PC	Gallic acid	−0.0427	−0.0123	0.0099	0.0545	0.0687
PC	4-Hydroxybenzoic acid	0.0692	0.0166	0.0041	−0.0588	**0.1035**
PC	Caffeic acid	0.0456	**0.1269**	**0.1313**	0.0075	0.0053
PC	Protocatechuic acid	0.0530	−0.0701	−0.0915	−0.0816	**0.1007**
PC	*p*-Coumaric acid	0.0271	0.0243	0.0272	−0.0085	0.0828
PC	Ferulic acid	−0.0497	0.0680	0.0954	0.0866	−0.0307
PC	Sinapic acid	−0.0917	−0.0773	−0.0626	0.0614	−0.0694
PC	(−)-Epicatechin	0.0233	0.0396	0.0370	−0.0089	−0.0022
PC	(+)-Catechin	−0.0395	0.0254	0.0507	0.0660	0.0505
PC	Hesperidin	**0.1003**	−0.0252	−0.0560	**−0.1144**	**0.1110**
PC	Genistein	0.0513	**0.1051**	**0.1070**	−0.0053	0.0358
PC	Daidzein	0.0911	0.0583	0.0421	−0.0677	0.0837
PC	Rutin	0.0767	0.0238	0.0089	−0.0651	**0.1070**
PC	Quercetin	−0.0244	0.0690	0.0863	0.0566	−0.0348
PC	Kaempferol	0.0940	**0.1153**	0.0993	−0.0548	0.0082
SAP	Phaseoside I	−0.0036	0.0325	0.0466	0.0290	0.2642
SAP	Soysaponin Bb (I)	0.0808	−0.0190	−0.0476	**−0.1067**	**0.1039**
SAP	Soysaponin βg	0.0878	−0.0175	−0.0544	**−0.1118**	0.0011
SAP	Soysaponin αg	**0.1079**	−0.0087	−0.0487	**−0.1271**	0.0305
PST	Fucosterol	0.0809	0.0732	0.0495	−0.0667	−0.0424
PST	β-Sitosterol	**−0.1061**	0.0451	0.0897	0.1411	−0.0394
PST	β-Campesterol	**−0.1254**	0.0187	0.0661	0.1511	−0.0401
PST	Δ7-Avenasterol	0.0215	**−0.1152**	**−0.1356**	−0.0706	0.0667
PST	Campesterol 3-β-glucopyranoside	0.0178	−0.0862	−0.0959	−0.0469	**0.1058**
PST	Stigmasterol 3-β-glucopyranoside	0.0737	0.0634	0.0526	−0.0477	0.0628

[a] Model coefficients for centered and scaled data. Phenolic compound (PC), phytosterol (PST), saponin (SAP), body weight (BW), triacylglycerides (TAG), total cholesterol (TC), low-density lipoprotein (LDL), lipase (LIP). Coefficient values in bold letters are significant.

and campesteryl 3-β-D-glucopyranoside (0.1058).

Accordingly, protocatechuic acid, hydroxybenzoic acid, rutin, and hesperidin are potent inhibitors of pancreatic lipase (Chen, Li, & Zhang, 2017; Karamać & Amarowicz, 1996; Martins et al., 2010; McDougall et al., 2009), and it has been reported that the lipase inhibitory activity of polyphenols depends on the number and position of hydroxyl groups and their polymerization degree (Buchholz & Melzig, 2015). There are no reports about the effect of soysaponin I and campesteryl 3-β-D-glucopyranoside on pancreatic lipase inhibition; nevertheless, it has been reported that saponins are inhibitors of pancreatic lipase activity (Marrelli et al., 2016; Singh et al., 2015).

3.3. Effect of selected phytochemicals associated with the hypotriglyceridemic effect of common bean sprouts on lipase enzyme activity through a molecular docking simulation

The phytochemicals associated with the inhibitory activity of common bean sprouts against pancreatic lipase according to the PLS-DA model coefficients (Table 3) were selected to carried out a molecular docking simulation against this enzyme, which were (code; Pubchem CID): 4-hydroxybenzoic acid (OHBA; 135), protocatechuic acid (PCA; 72), hesperidin (HPN; 10621), rutin (RTN; 5280805), soyasaponin I (SSI; 122097), and campesteryl 3-β-glucopyranoside (C3BG; 70699334).

According to Table 4, three subgroups with distinctive molecular features such as molecular weight, octanol/water partition coefficient and Lipinski's "rule of five" violations (MW/cLogP/LPV) were identified: Group A: OHBA, PCA (low/moderate/none), Group B: C3BG (high/high-very high/two) and, Group C: RTN, HPN, SSI (high/low-moderate/three). The higher MW is according to the higher values of topological polar surface area (TPSA), hydrogen bond-donor (HBDC), hydrogen bond-acceptor (HBAC), rotable bond (RBC) and LPV ($r = 0.87–0.95$) but not with cLogP ($r = 0.03$). Accordingly, TPSA, HBDC, and HBAC directly correlate with passive absorption of a

Table 4
Molecular informatics of selected phytochemicals of common bean sprouts.

Parameters	OHBA	PCA	C3BG	RTN	HPN	SSI
Molecular features						
Molecular weight (g/mol)	138.1	154.1	562.8	610.5	610.6	943.1
Octanol/water partition coefficient (cLogP)	1.4	0.9	6.7	−1.1	−0.6	1.7
Topological polar surface area (Å)	57.5	77.8	99.4	269.4	234.3	295.0
Hydrogen bond-donor (#)	2	3	4	10	8	11
Hydrogen bond-acceptor (#)	3	4	6	16	15	18
Rotable bond (#)	1	1	8	6	7	9
Lipinski's "rule of five" violations (#)	0	0	2	3	3	3
Molecular docking[a]						
ΔG_{bind} (Ligand- rat lipase; kcal/mol)	−5.8	−6.0	−7.6	−8.6	−8.5	−9.0
Druglikeenes[b]						
G protein-coupled receptors ligand	−0.98	−0.88	0.15	−0.05	−0.01	−3.24
Ion channel modulator	−0.40	−0.40	−0.20	−0.50	−0.60	−3.70
Kinase inhibitor	−1.21	−1.10	−0.40	−0.14	−0.36	−3.68
Nuclear receptor ligand	−0.62	−0.58	0.35	−0.23	−0.20	−3.52
Protease inhibitor	−1.19	−1.09	0.07	−0.07	0.00	0.06
Enzyme inhibitor	−0.41	−0.34	0.45	0.12	0.06	−3.16

Phytochemicals (code; Pubchem CID): 4-hydroxybenzoic acid (OHBA; 135), protocatechuic acid (PCA; 72), campesteryl-3-β-glucopyranoside (C3BG; 70699334), rutin (RTN; 5280805), hesperidin (HPN; 10621), soyasaponin I (SSI; 122097).

[a] ΔG of binding between ligand and *Rattus norvergicus* lipase [SWISS-PROT: Pancreatic lipase-related protein (LIPR2_RAT P54318)].

[b] Bioactivity score (molinspiration online property calculation toolkit; http://www.molinspiration.com).

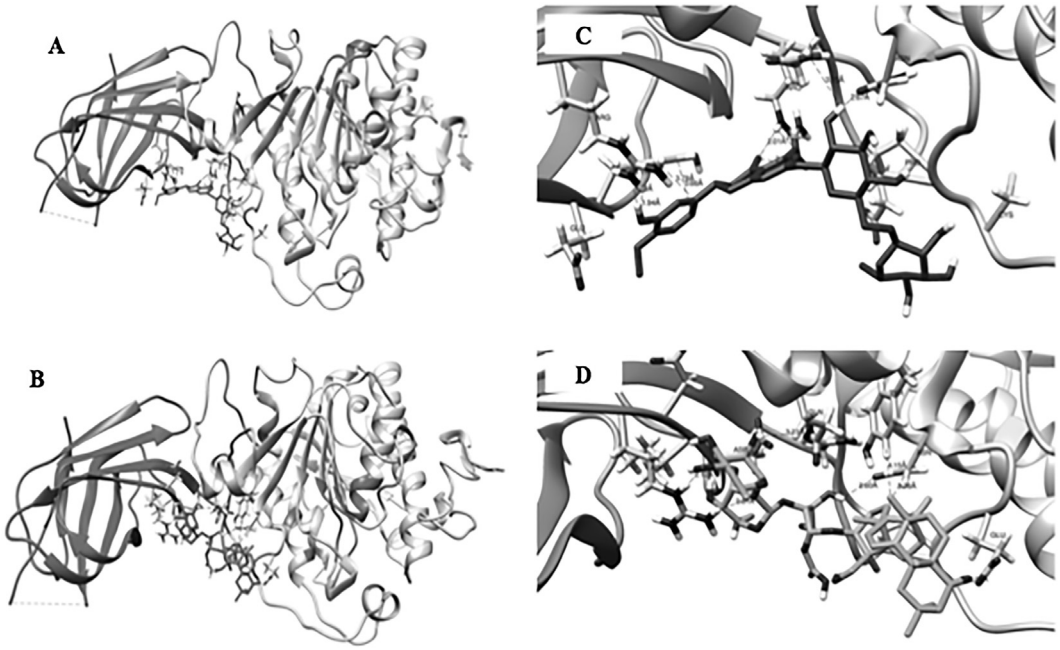

Fig. 3. Molecular docking between hesperidin (A, C) and soyasaponin I (B, D) and pancreatic lipase.

Table 5
Molecular docking data between rPLRP2 hesperidin and soyasaponin I.

Residue	Distance (Å)		Binding
	Hesperidin	Soyasaponin I	
Asn_{229}	2.67	2.6	Hydrogen bond
		3.8	Hydrophobic
Glu_{233}		3.1	Hydrogen bond
Cys_{237}	2.32		Hydrogen bond
Tyr_{280}		4.2	Hydrophobic
Gln_{292}		3.2	Hydrogen bond
Asn_{328}	3.15		Hydrogen bond
Arg_{337}	2.01		Hydrogen bond
	2.25		Hydrogen bond
	2.26		Hydrogen bond
Tyr_{369}	3.78		Hydrophobic
	3.66		Hydrophobic
Glu_{370}	2.33		Hydrogen bond
	2.52		Hydrogen bond
Arg_{384}	1.94	2.1	Hydrogen bond
	1.78	2.6	Hydrogen bond
Asp_{387}		3.3	Hydrogen bond

Summary of binding sites/distances depicted in Fig. 3.

particular molecule (Ertl, Rohde, & Selzer, 2000) but LPV, as it is influenced by MW and cLogP, may change this scenario.

A high clogP value means low hydrophilicity and poor permeation or absorption, therefore phytochemicals of group C could be less absorbed than those of Group A despite having a larger TPSA, which in turn would help to maintain their concentration for a longer time within the intestinal lumen (Gullón, Lú-Chau, Moreira, Lema, & Eibes, 2017). In addition, the number of rotatable bonds in a particular molecule is an important feature involved in all required conformational changes for binding to cell receptors or channels. In this sense, the conformational flexibility of the assessed molecules was higher for groups B and C as compare to the group A, but only C3BG may act as a nuclear ligand or as enzyme inhibitor according to its Molinspiration bioactivity scores (0.35, 0.45 respectively).

Computer-based molecular docking studies are often used to evaluate the binding mode of ligands toward a given protein. Detailed information on the nature (e.g. hydrophobic interactions, hydrogen bonding, etc.) and absolute binding force between an enzyme and a particular phytochemical is the starting point for designing potent or specific nutraceuticals for a given therapeutic purpose (Mohan, Gibbs, Cummings, Jaeger, & DesJarlais, 2005). The preferred ligand pose (molecular orientation/conformation) with the least binding energy (ΔG_{bind}) is often used as a scoring function to select the most plausible protein-ligand complex, therefore the most negative the ΔG_{bind} is the higher the probability of binding.

According to Table 4, all six phytochemicals showed negative ΔG_{bind} values for their interaction with rPLRP2, being those belonging to group C the most efficient (-8.5 to -9.0). These ΔG_{bind} values were comparable to those reported for human pancreatic lipase (hPTL; protein data bank 1LPB) and certain black tea flavonoids (-8.91 to -10.14) (Mohapatra et al., 2015). RTN (a flavonoid-O-diglycoside) is a well-studied lipase inhibitor (IC_{50} 57 μM; Orlistat IC_{50} 0.58 μM) and the molecular features for RTN-porcine pancreatic lipase adducts (π-π interaction with benzene ring of Phe-216) has been recently reported (Tao, Cai, Li, & Cai, 2015). These data could be similar for the interaction between RTN with hPLRP2 and even rPLRP2 due to their high homology with hPTL (Lowe, 2000).

The lower ΔG_{bind} values were observed for RTN, HPN and SSI, indicating that these phytochemicals showed a greater binding probability. Moreover, only HPN and SSI were associated with the hypotriglyceridemic effect of common bean sprouts as well as to their pancreatic lipase inhibitory activity. However, to the best of our knowledge, there is no information on docking studies involving PLRP2 (from any monogastric specie) and HPN or SSI.

rLIPR2 is a 1.8 Å protein (468 amino acids) with a globular (N-termini) and a β-sandwich (C-termini) domain, showing a 67% identity with rat pancreatic triglyceride lipase (rLIPP). It hydrolyzes (α/β serine-hydrolase) TAG (EC 3.1.1.3), phospholipids and galactolipids (EC 3.1.1.26) as LIPR2 from other species (Xiao, Ross, Sevilla, Wang, & Lowe, 2013), and requires interfacial activation by aggregated (e.g. oil drops, lipid bilayers or monomolecular lipid films) rather than monomeric substrates around its lid domain. Moreover, it is effectively inhibited by Orlistat (covalent inhibitor) as in case of human pancreatic lipase (hPTL), but do not seem to require co-lipase nor is inhibited by increasing bile salt concentration (Aloulou et al., 2006; Roussel et al., 1998).

hPTL active site is located at the bottom of a hydrophobic crevice covered by the "lid" (peptide stretch C238–C262) in its closed lid conformation which differ at positions 245, 257 y 258 when compared to rLIPP which presents an open lid conformation (Yang & Lowe, 2000). Its catalytic triad (Ser152, His263, and Asp176) includes the nucleophile belonging to the usual consensus sequence G-X-(nucleophile)-X-G (Roussel et al., 1998).

Fig. 3 depicts the binding modes between rPLRP2 and HPN (A, C) or SSI (B, D). According to Table 5, HPN has more binding residues (n = 12; distances between 1.8 and 3.8 Å) than SSI (n = 8; distances between 2.1 and 4.2 Å) outside the lid region of hydrogen bonding and hydrophobic nature.

Although HPN and SSI share three potential binding sites in rPLRP2 at Asn229 and Arg384, they differed in all other binding sites, which in turn could mean that both phytochemicals will not establish any competence with each other when present in an equimolar basis. Based on this data, and although kinetic studies have not been reported yet, HPN and SSI may interfere with the required conformational change and stability of rPLRP2 (interfacial inhibition) in the presence of lipids and amphiphiles (Aloulou et al., 2006). These results indicate that these phytochemicals could be acting synergistically on the hypotriglyceridemic effect of SA elicited common bean sprouts by acting as pancreatic lipase inhibitors.

4. Conclusion

Common bean sprouts exert antiobesogenic and hypolipidemic effects in obese rats. Elicitation with chitosan and salicylic acid improves the hypotriglyceridemic effect of common bean sprouts. The increased beneficial effect of SA-elicited common bean sprouts was associated with an increased TAG fecal excretion and pancreatic lipase inhibition. The combined approach of chemometrics and molecular docking studies allowed the identification of hesperidin and soysaponin I as the main inhibitors of lipase pancreatic activity. Therefore, SA-elicited common bean sprouts could be an alternative for the production of lipase inhibitors.

5. Declarations of interest

None.

6. Ethics statement

All animal experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

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Conflict of interest

Authors declare there are no conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

Acosta-Gallegos, J. A., Montero-Tavera, V., Jiménez-Hernández, Y., Anaya-López, J. L., & Gonzalez-Chavira, M. M. (2014). 'Dalia', nueva variedad de frijol de grano tipo Flor de Junio para la región centro de México. *Revista mexicana de ciencias agrícolas, 5*(2), 331–336. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2007-.

Aloulou, A., Rodriguez, J. A., Fernandez, S., van Oosterhout, D., Puccinelli, D., & Carrière, F. (2006). Exploring the specific features of interfacial enzymology based on lipase studies. *BBA-Molecular and Cell Biology of Lipids, 1761*(9), 995–1013. https://doi.org/10.1016/j.bbalip.2006.06.009.

Buchholz, T., & Melzig, M. F. (2015). Polyphenolic compounds as pancreatic lipase inhibitors. *Planta Medica, 81*(10), 771–783. https://doi.org/10.1055/s-0035-1546173.

Buettner, R., Schölmerich, J., & Bollheimer, L. C. (2007). High-fat diets: Modeling the metabolic disorders of human obesity in rodents. *Obesity, 15*(4), 798–808. https://doi.org/10.1038/oby.2007.608.

Chávez-Santoscoy, R. A., Tovar, A. R., Serna-Saldivar, S. O., Torres, N., & Gutiérrez-Uribe, J. A. (2014). Conjugated and free sterols from black bean (*Phaseolus vulgaris* L.) seed coats as cholesterol micelle disruptors and their effect on lipid metabolism and cholesterol transport in rat primary hepatocytes. *Genes & Nutrition, 9*(1), 367. https://doi.org/10.1007/s12263-013-0367-1.

Chavez-Santoscoy, R. A., Tovar, A., Granados, O., Ortiz-Ortega, V. M., Torres, N., Serna-Saldivar, S., & Gutiérrez-Uribe, J. A. (2013). Effects of extracts rich in phytosterols, flavonoids and saponins from black bean (*Phaseolus vulgaris* L.) seed coats on gene expression involved in {beta}-oxidation and lipogenesis. *The FASEB Journal, 27*(1_MeetingAbstracts), lb380. https://www.fasebj.org/doi/abs/10.1096/fasebj.27.1_supplement.lb380.

Chen, T., Li, Y., & Zhang, L. (2017). Nine different chemical species and action mechanisms of pancreatic lipase ligands screened out from *Forsythia suspensa* leaves all at one time. *Molecules, 22*(5), 795. https://doi.org/10.3390/molecules22050795.

Donangelo, C. M., Trugo, L. C., Trugo, N. M. F., & Eggum, B. O. (1995). Effect of germination of legume seeds on chemical composition and on protein and energy utilization in rats. *Food Chemistry, 53*(1), 23–27. https://doi.org/10.1016/0308-8146(95)95781-Z.

Ertl, P., Rohde, B., & Selzer, P. (2000). Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. *Journal of Medicinal Chemistry, 43*(20), 3714–3717. https://doi.org/10.1021/jm000942e.

Gorelick, J., & Bernstein, N. (2014). Elicitation: An underutilized tool in the development of medicinal plants as a source of therapeutic secondary metabolites. In D. L. Sparks (Vol. Ed.), *Advances in Agronomy: Vol. 124*, (pp. 201–230). Amsterdam: Elsevier. https://doi.org/10.4103/0973-1296.185726.

Gullón, B., Lú-Chau, T. A., Moreira, M. T., Lema, J. M., & Eibes, G. (2017). Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. *Trends in Food Science & Technology, 67*, 220–235. https://doi.org/10.1016/j.tifs.2017.07.008.

Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E., & Hutchison, G. R. (2012). Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics, 4*(1), 17. https://doi.org/10.1186/1758-2946-4-17.

Jakobek, L. (2015). Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chemistry, 175*, 556–567. https://doi.org/10.1016/j.foodchem.2014.12.013.

Kahlon, T. S., Smith, G. E., & Shao, Q. (2005). In vitro binding of bile acids by kidney bean (*Phaseolus vulgaris*), black gram (*Vigna mungo*), bengal gram (*Cicer arietinum*) and moth bean (*Phaseolus aconitifolins*). *Food Chemistry, 90*(1–2), 241–246. https://doi.org/10.1016/j.foodchem.2004.03.046.

Karamać, M., & Amarowicz, R. (1996). Inhibition of pancreatic lipase by phenolic acids-examination in vitro. *Zeitschrift für Naturforschung C, 51*(11–12), 903–906 PMID:9031529.

Kim, S., Hong, J., Jeon, R., & Kim, H. S. (2016). Adzuki bean ameliorates hepatic lipogenesis and proinflammatory mediator expression in mice fed a high-cholesterol and high-fat diet to induce nonalcoholic fatty liver disease. *Nutrition Research, 36*(1), 90–100. https://doi.org/10.1016/j.nutres.2015.11.002.

Klop, B., Elte, J. W. F., & Cabezas, M. C. (2013). Dyslipidemia in obesity: Mechanisms and potential targets. *Nutrients, 5*(4), 1218–1240. https://doi.org/10.3390/nu5041218.

Lin, P. Y., & Lai, H. M. (2006). Bioactive compounds in legumes and their germinated products. *Journal of Agricultural and Food Chemistry, 54*(11), 3807–3814. https://doi.org/10.1021/jf060002o.

López-Amorós, M. L., Hernández, T., & Estrella, I. (2006). Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition and Analysis, 19*(4), 277–283. https://doi.org/10.1016/j.jfca.2004.06.012.

Lowe, M. E. (2000). Properties and function of pancreatic lipase related protein 2. *Biochimie, 82*(11), 997–1004. https://doi.org/10.1016/S0300-9084(00)01184-6.

Martins, F., Noso, T. M., Porto, V. B., Curiel, A., Gambero, A., Bastos, D. H., ... Carvalho, P. D. O. (2010). Maté tea inhibits in vitro pancreatic lipase activity and has hypolipidemic effect on high-fat diet-induced obese mice. *Obesity, 18*(1), 42–47. https://doi.org/10.1038/oby.2009.189.

Marrelli, M., Conforti, F., Araniti, F., & Statti, G. A. (2016). Effects of saponins on lipid metabolism: A review of potential health benefits in the treatment of obesity. *Molecules, 21*(10), 1404. https://doi.org/10.3390/molecules21101404.

McDougall, G. J., Kulkarni, N. N., & Stewart, D. (2009). Berry polyphenols inhibit pancreatic lipase activity in vitro. *Food Chemistry, 115*(1), 193–199. https://doi.org/10.1016/j.foodchem.2008.11.093.

Mendoza-Sánchez, M., Guevara-González, R. G., Castaño-Tostado, E., Mercado-Silva, E. M., Acosta-Gallegos, J. A., Rocha-Guzmán, N. E., & Reynoso-Camacho, R. (2016). Effect of chemical stress on germination of cv Dalia bean (*Phaseolus vularis* L.) as an

alternative to increase antioxidant and nutraceutical compounds in sprouts. *Food Chemistry, 212*, 128–137. https://doi.org/10.1016/j.foodchem.2016.05.110.

Mohan, V., Gibbs, A. C., Cummings, M. D., Jaeger, E. P., & DesJarlais, R. L. (2005). Docking: Successes and challenges. *Current Pharmaceutical Design, 11*(3), 323–333. https://doi.org/10.2174/1381612053382106.

Mohapatra, S., Prasad, A., Haque, F., Ray, S., De, B., & Ray, S. S. (2015). In silico investigation of black tea components on α-amylase, α-glucosidase and lipase. *Journal of Applied Pharmaceutical Science, 5*(12), 42–47. https://doi.org/10.7324/JAPS.2015.501207.

Nilsson, A., Johansson, E., Ekström, L., & Björck, I. (2013). Effects of a brown beans evening beal on metabolic risk markers and appetite regulating hormones at a subsequent standardized breakfast: A randomized cross-over study. *PLoS ONE, 8*(4), e59985. https://doi.org/10.1371/journal.pone.0059985.

Pérez-Ramírez, I. F., González-Dávalos, M. L., Mora, O., Gallegos-Corona, M. A., & Reynoso-Camacho, R. (2017). Effect of *Ocimum sanctum* and *Crataegus pubescens* aqueous extracts on obesity, inflammation, and glucose metabolism. *Journal of Functional Foods, 35*, 24–31. https://doi.org/10.1016/j.jff.2017.05.028.

Ramírez-Jiménez, A. K., Reynoso-Camacho, R., Tejero, M. E., León-Galván, F., & Loarca-Piña, G. (2015). Potential role of bioactive compounds of *Phaseolus vulgaris* L. on lipid-lowering mechanisms. *Food Research International, 76*(P1), 92–104. https://doi.org/10.1016/j.foodres.2015.01.002.

Roussel, A., Yang, Y., Ferrato, F., Verger, R., Cambillau, C., & Lowe, M. (1998). Structure and activity of rat pancreatic lipase-related protein 2. *Journal of Biological Chemistry, 273*(48), 32121–32128. https://doi.org/10.1074/jbc.273.48.32121.

Singh, G., Suresh, S., Bayineni, V. K., & Kadeppagari, R. K. (2015). Lipase inhibitors from plants and their medical applications. *International Journal of Pharmacy and Pharmaceutical Sciences, 7*(1), 1–5 ISSN: 0975-1491.

Spadafranca, A., Rinelli, S., Riva, A., Morazzoni, P., Magni, P., Bertoli, S., & Battezzati, A. (2013). Phaseolus vulgaris extract affects glycometabolic and appetite control in healthy human subjects. *British Journal of Nutrition, 109*(10), 1789–1795. https://doi.org/10.1017/S0007114512003741.

Stone, N. J., Robinson, J. G., Lichtenstein, A. H., Merz, C. N. B., Blum, C. B., Eckel, R. H., ... Wilson, W. P. F. (2014). 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Journal of the American College of Cardiology, 63*(25 Part B), 2889–2934. https://doi.org/10.1161/01.cir.0000437738.63853.7a/-/DC.

Świeca, M., & Baraniak, B. (2014). Influence of elicitation with H2O2 on phenolics content, antioxidant potential and nutritional quality of *Lens culinaris* sprouts. *Journal of the Science of Food and Agriculture, 94*(3), 489–496. https://doi.org/10.1002/jsfa.6274.

Tao, Y., Cai, H., Li, W., & Cai, B. (2015). Ultrafiltration coupled with high-performance liquid chromatography and quadrupole-time-of-flight mass spectrometry for screening lipase binders from different extracts of Dendrobium officinale. *Analytical and Bioanalytical Chemistry, 407*(20), 6081–6093. https://doi.org/10.1007/s00216-015-8781-4.

Tappy, L., & Lê, K. A. (2010). Metabolic effects of fructose and the worldwide increase in obesity. *Physiological Reviews, 90*(1), 23–46. https://doi.org/10.1152/physrev.00019.2009.

Teodoro, J. S., Varela, A. T., Rolo, A. P., & Palmeira, C. M. (2014). High-fat and obesogenic diets: Current and future strategies to fight obesity and diabetes. *Genes & Nutrition, 9*(4), 406. https://doi.org/10.1007/s12263-014-0406-6.

Tippel, J., Gies, K., Harbaum-Piayda, B., Steffen-Heins, A., & Drusch, S. (2017). Composition of *Quillaja saponin* extract affects lipid oxidation in oil-in-water emulsions. *Food Chemistry, 221*, 386–394. https://doi.org/10.1016/j.foodchem.2016.10.055.

Xiao, X., Ross, L. E., Sevilla, W. A., Wang, Y., & Lowe, M. E. (2013). Porcine pancreatic lipase related protein 2 has high triglyceride lipase activity in the absence of colipase. *Biochimica et Biophysica Acta, 1831*(9), 1435–1441. https://doi.org/10.1016/j.bbalip.2013.06.00.

Yang, Y., & Lowe, M. E. (2000). The open lid mediates pancreatic lipase function. *Journal of Lipid Research, 41*(1), 48–57 PMID:10627501.