Protein hydrolysates and phenolic compounds from fermented black beans inhibit markers related to obesity and type-2 diabetes

Sergio A. Flores-Medellín1,4 | Rosa M. Camacho-Ruiz2 | Cecilia Guízar-González3 | Edgar A. Rivera-Leon4 | Iris M. Llamas-Covarrubias4 | Luis Mojica1

1Tecnología Alimentaria, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A. C., CIATEJ, Zapopan, México
2Biotecnología Industrial, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A. C., CIATEJ, Zapopan, México
3Biotecnología Vegetal, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A. C., CIATEJ, Zapopan, México
4Departamento de Biología Molecular y Genómica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, México

Correspondence
Luis Mojica, Tecnología Alimentaria, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A. C., CIATEJ, Camino Arenero 1227, El Bajío del Arenal, 45019, Zapopan, Jalisco, México.
Email: lmojica@ciatej.mx

Abstract
Obesity and type 2 diabetes mellitus (T2DM) represent an epidemic and public health problem. Bioactive compounds from foods could be used as adjuvants in the prevention of these diseases. Processes such as solid-state fermentation (SSF) have demonstrated to improve the release of bioactive compounds from food matrix. This project aimed to evaluate the effect of fermented black bean (Phaseolus vulgaris L.) phenolic compounds and protein hydrolysates on markers associated with obesity and T2DM. Twenty-eight peptide sequences and 12 phenolic compounds were identified on black bean samples. From in silico assays, sequenced peptides showed theoretical binding energies up to −9.8 kcal/mol and phenolic compounds up to −10.1 kcal/mol for monoacylglycerol lipase. Protein hydrolysates from SSF 96 h (12 peptides) showed the potential to inhibit α-amylase with inhibitory concentration 50 (IC50) 0.57 mg protein/ml and 5.55 mg protein/ml for α-glucosidase. Phenolic compounds from SSF 48 h blocked α-glucosidase IC50 0.353 mg GAE/ml. The antioxidant capacity was maintained in phenolic compounds after fermentation for 48 h and improved in the protein hydrolysates. Raw bean protein hydrolysates (0.1 mg protein/ml) presented the potential to inhibit lipid accumulation in 3T3-L1 cell line (27.9%). SSF is a processing method for generating functional ingredients rich in bioactive compounds capable of acting on biological markers related to the treatment or prevention of obesity and T2DM.

KEYWORDS
bioactive peptides, biological potential, black beans, phenolic compounds

1 | INTRODUCTION

Obesity is a chronic condition characterized by inflammation and maturation of adipocytes; therefore, the accumulation of fatty acids, excess of body weight, and release of proinflammatory cytokines like tumor necrosis factor (TNF α) and interleukins (IL 6, IL 8, IL 10) (Gadde, Martin, Berthoud, & Heymsfield, 2018; Gnacińska, Małgorzewicz, Stojek, Łysiak-Szydlowska, & Sworczak, 2009; Graßmann, Wirsching, Eichelmann, & Aleksandrova, 2017; Pereira & Alvarez-Leite, 2014) trigger diseases like type-2 diabetes mellitus (T2DM).
T2DM is defined as a metabolic disorder characterized by progressive loss of insulin secretion and insulin resistance, generate high blood glucose levels, and its etiology is not specific. Pharmacotherapy includes thiazolidinediones (TZDs), which are activators of proliferator-activated receptor gamma (PPAR-γ), dipeptidyl peptidase-IV inhibitors (DPP-IV), which maintains the activity of incretins, and α-glucosidase and α-amylase inhibitors that produce slow intestinal carbohydrate digestion (ADA, 2019). As adjuvants to pharmacotherapy, bioactive compounds have demonstrated to modulate glucose homeostasis, suppression of inflammation factors like TNF-α and IL-6, and inhibition of α-amylase and α-glucosidase enzymes (Azzini, Giacometti, & Russo, 2017; Mojica, Berhow, Gonzalez, & Mejia, 2017; Mojica, Gonzalez de Mejia, Granados-Silvestre, & Menjivar, 2017). Foods such as black beans (Phaseolus vulgaris L.) contain bioactive components including polyphenols and bioactive peptides, which could act on T2DM molecular markers (Mojica, Berhow, et al., 2017; Mojica, Luna-vital, González, & Mejia, 2016).

Bioactive peptides are secondary metabolites of plants; they are formed mainly of an aromatic ring with one or more hydroxyl groups, and the bioactive compounds are responsible for the color of red fruits and juices and are involved in flavor properties, important characteristics for the quality of plant-based foods (Chavez-servia, Aquino-bolaños, García-diaz, & Chavez-servia, 2016; Sánchez, Cruz-martín, Sánchez-garcía, & Leiva-mora, 2016). This family of molecules presents antioxidant capacity and various beneficial effects in the prevention and treatment of noncommunicable diseases. (Guo, Guo, Jiang, Li, & Ling, 2012; Luna-Vital, Weiss, & Gonzalez de Mejia, 2017).

Bioactive peptides are sequences of amino acids that are found within the primary structure of proteins, generated from the hydrolysis of protein, usually by proteolytic enzymes, which have shown bioactive compounds including polyphenols and bioactive peptides, which could act on T2DM molecular markers (Mojica, Berhow, et al., 2017; Mojica, Luna-vital, González, & Mejia, 2016).

Processes such as solid-state fermentation (SSF) have the potential to increase the release of bioactive compounds from the food matrix. During SSF, enzymes hydrolyze trypsin inhibitors, phytic acids among other antinutritional components from the seeds, increasing their protein value. Furthermore, Bacillus subtilis have shown an enhanced content of antioxidant potential of beans (Dey, Chakraborty, Jain, Sharma, & Kuhad, 2016) and generation of peptides with antioxidant potential (He et al., 2012). The objective of this work was to evaluate the effect of SSF with B. subtilis on black beans proteins and phenolic compounds and their potential for inhibiting markers associated with obesity and T2DM.

2 | MATERIALS AND METHODS

The samples of black bean were acquired at a local market, 2017 harvest. Black beans were cleaned to remove foreign material and damaged seeds. DC protein assay and SDS-PAGE were acquired from BIORAD, California, USA. NaOH, HCl, phosphate buffer saline (PBS), picrylsulfonic acid (TNBS), Folin–Ciocalteu 2N, gallic acid >99%, Na2CO3, potassium chloride buffer, sodium acetate buffer, quercetin standard >99%, NaNO2, AlCl3, catechin standard 99.9%, methanol, vanillin 99.9%, α-amylase, α-glucosidase, acarbose, p-nitrophenyl-α-D-glucopyranoside, soluble starch, dinitrosaliclycic acid, and 2′-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) were acquired from Sigma-Aldrich, Missouri, USA.

2.1 | Processing and characterization

Black bean samples were analyzed as raw, cooked, and fermented. Phenolic compounds and protein were extracted. Protein isolates were hydrolyzed using a simulated gastrointestinal digestion assay (pepsin and pancreatin). Phenolic compounds extraction was performed using acidified ethanol. Phenolic extracts and protein hydrolysates were fully characterized. The biological potential was evaluated using in silico, biochemical and in vitro assays (Figure 1).

2.1.1 | Raw and cooked beans

Black beans (Phaseolus vulgaris L.) were acquired from the local market and cleaned before use. Black beans were used as cooked and uncooked controls. For cooked control, black beans were suspended in purified water (1:6 w/v) and cooked for 70 min in a pressure cooker at low heat. The sample was dried using a convection oven at 60°C, milled and sieved using 40 mesh, and stored at −20°C until use. For raw control, black beans were milled, sieved using 40 mesh, and stored at −20°C until use.

2.1.2 | Solid-state fermentation

B. subtilis was cultivated in Luria-Bertani broth at 30°C for 24 h. The microorganisms were counted using a Neubauer chamber adjusted to a concentration of 10⁷ cells/ml in sterile distilled water. The B. subtilis suspension was used to inoculate chopped and sterilized black beans and start the fermentation. SSF was carried out in stainless steel trays in a controlled chamber. After the incubation time, samples were collected, freeze-dried, milled, sieved using 40 mesh, and stored at −20°C until use.

2.1.3 | Protein extraction

Protein extraction was carried out following Mojica, Chen, and de Mejia (2015). Fermented bean, raw bean, and cooked bean samples were suspended at a 1:10 bean/water ratio, pH 8.0, adjusted with
0.1 M NaOH, and temperature of the 35°C in constant agitation for protein extraction during 1 h. The mix was centrifuged at 4,000 RPM for 15 min at 25°C. The supernatant was adjusted to pH 4.3 with 0.1 M HCl to precipitate proteins, followed by centrifugation at 4,000 RPM for 15 min at 4°C. The liquid supernatant was removed and consequently discarded, the pellet was freeze-dried, and common bean protein isolates (BPI) were stored at −20°C for further analysis.

2.1.4 | Soluble protein

Soluble protein was determined using the DC protein assay kit (BIO-RAD). Five microliters of hydrolysate or blank was added in a 96-well plate with 25 μl reagent A and 200 μl reagent B, incubated for 15 min, and read at 690 nm. Protein concentration was calculated using a bovine serum albumin standard curve (10–1,200 μg/ml).

2.1.5 | Protein hydrolysis

Protein hydrolysis was performed using simulated gastrointestinal digestion (GI) following the method reported by Mojica, Chen, and de Mejía (2015). Protein was incorporated in distilled water (1:20 w/v) and solubilized by sonication for 15 min. Hydrolysis was carried out with pepsin in ration 1:20 (weight enzyme/dry weight protein), for 2 h, at 37°C, and pH 2.0 was adjusted with 0.1 N HCl. Subsequently, pancreatin was added in ration 1:20 (weight enzyme/dry weight protein), for 2 h, 37°C, and pH 7.5, which was adjusted with 0.1 N NaOH. Enzymes were inactivated in a water bath at 70°C for 20 min. Hydrolysates were centrifuged at 4,000 RPM for 15 min; the supernatant was freeze-dried and stored at −20°C.

2.1.6 | Electrophoresis

SDS-PAGE for protein and protein hydrolysates profile were performed following manufacturer instructions (BIORAD, California, USA) using reducing conditions (5% β-mercaptoethanol in Laemmli buffer). Gels were run at 200 V for 35 min. Molecular weight standard 10 to 250 kDa was used. SimplyBlue SafeStain (ThermoFisher, Massachusetts) was used for visualizing the bands. Gels were visualized using a Gel Doc™ EZ Gel Documentation System (BioRad, USA).

2.1.7 | Degree of hydrolysis

The degree of hydrolysis (DH) was carried out according to San Pablo-Osorio, Mojica, and Urias-Silvas (2019). Sixty-four microliters of the samples were added to 1 ml of 0.2125 M phosphate buffer, pH 8.2, 0.05% picrylsulfonic acid (TNBS) in light-protected tubes and incubated for 30 min in a water bath at 50°C, the reaction was stopped by adding 0.1 M sodium sulfite. It remained for 15 min at room temperature, and the absorbance was read at 420 nm. The total hydrolysis of the sample was carried out with 6 N HCl at 110°C for 24 h. A calibration curve was performed using leucine (0–10 mM) as standard. DH was calculated according to the following equation:

\[
\%DH = \left( \frac{h}{h_{total}} \right) \times 100.
\]

where h is the number of free amino groups in the hydrolyzed sample and h_{total} is the number of free amino groups after complete hydrolysis of the raw bean.
2.1.8 | Peptides characterization and sequence identification by LC-ESI-MSMS

Protein hydrolysates were characterized by LC-ESI-MSMS using a QToF Ultima mass spectrometer 175 (Waters, Milford, USA), equipped with the system Alliance 2795 HPLC. The compounds separation was performed using a 2.1 mm ID reverse phase C-18 column. Two mobile phases of solvent were used, solvent A (5% ACN, 0.1% formic acid in ACN) and solvent B (5% H2O and 0.1% formic acid in H2O), in a flow rate of 0.20 μl/min. The elution was in a linear gradient (0 min, 100% A; 2 min, 90% A; 6 min, 60% A; 10 min, 0% A; 12 min, 0% A; 12.10 min, 100% A; 15 min, 100% A). A splitter with a split ratio of 1:10 was used, where one part went to the mass spectrometer and 10 parts to the waste. The QToF Ultima mass spectrometer was equipped with a Z-spray ion source. Using a positive ion electrospray mode (+ESI), the analysis on the QToF was carried out in V-mode with an instrument resolution between 9,000 and 10,000 based on full width at half maximum, with a flow rate of 20 μl/min. The source temperature was set at 80°C, and desolvation temperatures were set at 250°C. The temperature was kept in 20°C during the whole procedure. The QToF was operated at a capillary voltage of 35 kV and a cone voltage of 35 V. The final detector was a microchannel plate with high sensitivity. The MassLynx 4.1 V software (Waters, Milford, USA) was used to control the instruments and to process the data to obtain the highest probability of the peptides sequences. BLAST® was used for confirmation of peptide sequences (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 06/1/2019).

2.1.9 | Extraction of phenolic compounds

Phenolic compounds from fermented, raw, and cooked black beans were extracted using the methodology reported by Mojica, Berhow, et al. (2017). Bean flours were suspended (1:40 w/v) in acidified ethanol solution (23% ethanol and 2% formic acid) and stirred during 2 h at 30 ± 5°C in beakers protected from light. After extraction samples were centrifuged 4,000 RPM for 15 min and 25°C, the supernatant was evaporated at 40°C using a vacuum evaporator and stored at −20°C until analysis.

2.1.10 | Total phenolic compounds

Total phenolic compounds from fermented, raw, and cooked black beans were quantified using the Folin–Ciocalteu method with some modifications (Johnson, Lucius, Meyer, & Mejia, 2011). To a 96-well plate, 50 μl (ethanol extract) of either bean polyphenols extracts, standard curve (40–200 μg gallic acid/ml) or blank were added with 50 μl of 1 N Folin–Ciocalteu’s phenol reagent. The mixture was allowed to stand for 5 min and subsequently was added 100 μl of 20% Na2CO3. The solution was allowed to stand for 10 min before reading the absorbance at 690 nm. The results were expressed as mg of gallic acid (GA) equivalents per gram of bean.

2.1.11 | Total anthocyanins

Total anthocyanins from fermented, raw, and cooked black beans were determined using the AOAC Official Method (2005.02). Samples (Section 2.1.9) were diluted to a factor of 1:10 using two buffers (pH 1.0, 0.025 M potassium chloride buffer and pH 4.5, 0.4 M sodium acetate buffer). A 200 μl of diluted solutions was transferred to a 96-well plate, and absorbance was read at 520 and 700 nm. The total anthocyanins were expressed as mg of cyanidin-3-glucoside (C3G) equivalents per gram of bean.

\[
\text{Total anthocyanin} = \frac{\mu l \text{ of } [\text{A} \times 1.7] - \mu l \text{ of } [\text{A} \times 3.01]}{\mu l \text{ of } 1 N \text{ NaOH} + 60 \mu l \text{ distilled water}} \times 2 + DF \times 1,000 \times 26,900
\]

where A is the absorbance and DF is the dilution factor.

2.1.12 | Total flavonoids

Samples (Phenolic compounds from fermented, raw, and cooked black beans) (125 μl) and the quercetin standard curve (50-350 μg/ml) were added to a 96-well plate. Then 7.5 μl 5% NaNO2 was added and incubated for 5 min at room temperature. Next, 7.5 μl of 10% AlCl3 was added and incubated for 6 min. Finally, 50 μl 1 N NaOH and 60 μl distilled water were added to each well and the absorbance was read at 510 nm. Results were expressed as mg quercetin equivalents (QE) per gram of bean (Mojica, Meyer, Berhow, González, & Mejia, 2015).

2.1.13 | Total tannins

The method used for total tannins was based on Mojica, Meyer, et al. (2015). Fifty microliters of phenolic extracts sample (Section 2.1.9) or catechin standard (0.1–0.8 mg/ml) were added to a 96-well plate, followed 200 μl of 8% acidified methanol and 1% vanillin 1:1 solution by 50 μl until complete 250 μl was added. Blank is makeup to 50 μl of methanol and 200 μl of 4% acidified methanol. Absorbance was read at 500 nm, using a Tecan infinite M200 pro. The concentration of condensed tannins was calculated and expressed as mg catechin equivalents (CAE) per gram of bean.

2.1.14 | Identification of phenolic compounds by ESI-QToF-MS

The 12 reference standards and the methanol extracts were analyzed by direct injection into the TOF mass spectrometer following the methodology of Chen, Wortmann, Zhang, and Zenobi (2007) with some modifications. The mass spectra were acquired using a Xevo-G2-S QToF mass spectrometer (TOF-MS, Waters, UK) equipped with an ESI interface. The optimized mass spectrometric parameters were as follows: in positive ion mode (ESI+), capillary voltage 3,000 V, source temperature 150°C, desolvation temperature...
450°C, desolvation gas 900 L/h, gas flow in the cone 50 ml/min, and the flow of the sample 5 μl/min; in negative ion mode (ESI−), capillary voltage 2,500 V, source temperature 130°C, desolvation temperature 500°C, desolvation gas 1,000 L/h, gas flow in the cone 50 ml/min, and the flow of the sample 5 μl/min. The mass scale was calibrated using the calibration solution provided by the manufacturer between m/z 50 and 1,500 Da. The protonated molecule [M + H]+ or deprotonated molecule [M – H]− was selected as the precursor ion for CID fragmentation to produce MS/MS product ion spectra. The most prominent ion product was then selected for further MS analysis. The collision energy was adjusted between 10% and 50%. The fragmentation pattern of reference standards and the samples were corroborated in the MassBank of North America (MoNA) http://mona.fiehnlab.ucdavis.edu/.

2.1.15 | Identification and quantification of anthocyanins by HPLC-DAD

In the case of anthocyanins, a sample of 15 μl of the crude acidic methanolic extract was injected in the HPLC-DAD system (1260 Infinity, Agilent Technologies, Santa Clara, CA, USA) equipped with a column analytical reverse-phase C-18 Zorbax SB rapid resolution (4.6 × 50 mm, 1.8 μm particle size). The mobile phase used was composed of 0.5% phosphoric acid in water (A) and 0.1% acetic acid in acetonitrile (B) with a flow rate of 1 ml/min; column temperature, 25°C; analysis time, 15 min; wavelength, 520 nm. In this case, the anthocyanin quantification was performed using a calibration curve for each anthocyanin. The concentration was expressed as μg of each anthocyanin individual per gram of dry tissue.

2.2 | In silico assays

Molecular interactions were between proteins, and bioactive compounds were predicted using molecular docking. The 3D crystal structure of α-amylase (1B2Y), α-glucosidase (3J7), peroxisome proliferator-activated receptor gamma (PPAR-γ) (5DSH), monoaclglycerol lipase (MGL) (3PE6), and phospholipase-A2 (PLA2) (5IBP) and 2D structure of acarbose, pioglitazone, catechin, gallic acid, rosmarinic acid, rutin, vanillin, quercetin, naringenin, malvidin, and cyanidin were acquired from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/).

Peptides sequences were drawn and 3D performed in MarvinSketch software version 17.29.0 and converted to PDB extension using the Discovery Studio Visualizer software version 17.2.0.16349. Flexible torsions, charges, and grid box size (20-30) were assigned using AutoDock Tools. Binding energies were performed using AutoDock Vina (Trott & Olson, 2010). Moreover, the binding pose with the lowest binding energy was selected as a representative to visualize in the Discovery Studio Visualizer software. Peptide physicochemical properties were predicted using the PepDraw tool (http://www.tulane.edu/?biochem/WW/PepDraw/), and protein sources were obtained from protein blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3 | Biological potential

2.3.1 | α-Amylase and α-glucosidase inhibition

The α-amylase and α-glucosidase assays were carried out following (Johnson et al., 2011). Fifty-microliters of samples, positive control (1 mM acarbose) or negative control (distilled water), were added to 50 μl of 13 U/ml α-amylase solution (type VI-B from porcine pancreas in 0.02 M sodium phosphate buffer, pH 6.9) or 100 μl of a 1-U/ml α-glucosidase solution (in 0.1 M sodium phosphate buffer, pH 6.9) and incubated at 37°C for 10 min. For the α-amylase assay, 50 μl of 1% soluble starch solution (previously dissolved in 0.02 M sodium phosphate buffer, pH 6.9 and boiled during 10 min) was added to each tube and then leave incubating for 10 min. Finally, 200 μl of dinitrosalicylic acid color reagent was added, and the tubes were placed in a boiling water bath for 5 min. The mixture was diluted with 1 ml of distilled water, and absorbance was read at 520 nm using a clear microplate. For the α-glucosidase assay, a 50 μl of 5-mM ρ-nitrophenyl-α-α-glucopyranoside solution (in 0.1 M sodium phosphate buffer, pH 6.9) was added and incubated at 37°C for 5 min; subsequently, the absorbance was read at 405 nm. For both assays, the results are presented as a percent of inhibition relative to the negative control.

% inhibition = \( \frac{\Delta \text{Absorbance control} - \Delta \text{Absorbance sample}}{\Delta \text{Absorbance control}} \times 100 \)

2.3.2 | ABTS

Seven-micromolar 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and potassium persulfate 2.45 mM were mixed at 25°C in the dark for 20 h. ABTS solution was adjusted with distilled water, and absorbance was read at 734 nm. In a 96-well plate, 20 μl of protein hydrolysates or phenolic compounds, blank or positive control (Trolox 0.01–0.3 mM), were added with 180 μl of the prepared ABTS solution and mixed thoroughly. The plate was read at 734 nm using the Tecan Infinite M200 pro (Hsieh-Lo, Castillo-Herrera, & Mojica, 2020).

2.3.3 | Cell culture and adipocyte differentiation

3T3-L1 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS), 1.5 g/L sodium bicarbonate, and 1% antibiotic penicillin/streptomycin, at 37°C with 5% CO2. Adipocytes differentiation was induced by some modifications in medium, DMEM for differentiation containing 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine, 0.25 μM dexamethasone, 1 μg/ml insulin, 2 μM rosiglitazone, 1.5 g/L sodium bicarbonate, and 1% antibiotic penicillin/streptomycin.
penicillium/streptomycin. On day 4, the medium was changed to DMEM containing 10% FBS and 1 μg/ml insulin, 1.5 g/L sodium bicarbonate, and 1% antibiotic penicillium/streptomycin. On day 7, the medium was changed to DMEM containing 10% FBS, 1.5 g/L sodium bicarbonate, and 1% antibiotic penicillium/streptomycin, and the cells were kept in this culture media until treatments.

2.3.4 Determination of viability

Cells were seeded in 96-well plate and induced differentiation as described in Section 2.3.3. Different concentration of phenolic compounds or protein hydrolysates was added; PBS was used as a blank and was incubated for 24 h. The medium was replaced with 80 μl of medium without SFB, and 20 μl MTT solution to a concentration of 1 mg/ml PBS was added then left incubated for 4 h in 96-well plate. The supernatant was removed, and formazan crystals were dissolved with 100 μl dimethyl sulfoxide (DMSO). Absorbance was read 540 nm, and viability was calculated as percentage respect to blank.

2.3.5 Determination of cellular lipid accumulation

Lipid accumulation assays were performed following the method reported by Luna-Vital et al. (2017). Cells were seeded in 96-well plate and induced differentiation as described in Section 2.3.3. Different concentration of phenolic compounds or protein hydrolysates were added in the differentiation process, PBS was used as a blank, and lipid staining was performed on day 10, with 0.35% Oil Red O in isopropanol. Cells were fixed using 10% formalin, and 100 μl solution Oil Red was added for 5 min and eluted with isopropanol 100% for final detection at 500 nm. Lipid accumulation was calculated as percentage respect to blank.

2.3.6 Reactive oxygen species

Reactive oxygen species assays were performed following the method reported by Mojica, Gonzalez de Mejia, et al. (2017). Adipocyte differentiation was realized in 96-well plates 5,000 cells as described in Section 2.3.3. Cells were treated with 100 μM H2O2, 25 μM 2,7-dichlorofluorescein diacetate (DCFDA), and phenolic compounds or peptides in different concentrations. Trolox curve was used as a reference. After 4 h of incubation at 37°C and 5% CO2, the plate was read with excitation wavelength at 485 nm and emission wavelength at 535 nm. Results were represented as IC50.

2.4 Statistical analysis

Statistical analysis was performed using SPSS v20. The data were obtained by least three independent replications, analyzed using analysis of variance (ANOVA) with Tukey test to identify significant differences among treatments, and the differences were considered significant at p < 0.05. The IC50 was calculated using GraphPad Prisma 7.04. Results are reported as a mean ± standard deviation.

3 RESULTS

3.1 Characterization

3.1.1 Protein profile

The undigested bean protein isolate (BPI) and the digested samples (protein hydrolysates) protein profiles were compared with the molecular standard 10–250 kDa in the electrophoresis gel (Figure 2). A consistent band around 40 kDa that corresponds to phaseolin (Mojica et al., 2016) is observed in all samples except in the cooked treatment with GI digestion. A band around 100 kDa corresponds to lipoxygenase 1 and disappears in fermented by 48 and 96 hr and cooked treatment with GI digestion. However, bands around 20–50 kDa appear in fermented by 48 and 96 h. A band around 50 kDa can be observed in undigested fermented samples after 48 and 96 hours. Besides, more intense bands can be observed in fermentation for 96 h at a molecular weight below 20 kDa.

3.1.2 Degree of hydrolysis

The degree of hydrolysis (DH) is represented in Figure 3. BPI DH ranged from 11.1% to 32.3% corresponding to raw black bean and cooked bean, respectively. SSF for 48 and 96 h showed DH values of 24.3% and 26.2%, respectively. Significant differences in DH are presented in raw bean compared with cooked and fermented samples. Protein hydrolysates with gastrointestinal (GI) digestion present higher values of DH, ranging from 39.8% to 75.1%. Significant differences are presented in raw bean and fermented 48 h, compared with cooked bean and fermented 96 h.

3.1.3 Peptides characterization and sequence identification by LC-ESI-MSMS

Identified peptide sequences are presented in Table 1. Twenty-eight peptides were identified in all samples: 5 in raw black bean, 7 in the cooked bean, 4 in fermented 48 h, and 12 in fermented 96 h. Peptides molecular mass ranged from 342.1 to 1,559.7 Da, isoelectric point ranged from 3.69 to 11.13, and hydrophobicity ranged from 6.41 to 25.54 kcal/mol. Peptide sequence confirmation was performed using blast analysis of parental protein from Phaseolus vulgaris including arcelin-1, leghemoglobin, lectin, phaseolin, profilin-1, lipoxygenase, and alpha-amylase inhibitor 1.
3.1.4 | Phenolic compounds content

The content of total polyphenols (Figure 4a) increased after the cooking and SSF process. However, significant differences compared to raw samples (17.75 mg GAE/g bean) were observed in fermented 96 h (25.39 mg GAE/g bean). Total flavonoids content (Figure 4b) is constant among treatments, showing no significant differences ranging from 5.33 to 7.53 mg QUE/g, in raw bean and fermented 96 h, respectively. Total anthocyanins content (Figure 4c) decreased during cooking and SSF processes, starting from 0.273 mg EC3G/g bean in raw samples to 0.022 mg EC3G/g bean in cooked samples and 0.01 mg and 0.005 mg EC3G/g in fermented 48 and 96 h, respectively. The total tannins (Figure 4d) ranged from 4.21 to 21.04 mg CAE/g bean. The tannins content presented a significant reduction after cooking and SSF (p < 0.05). Cooked and fermented 48 h samples presented similar values 4.21 and 4.67 mg CAE/g bean, respectively. The conditions during SSF (light and oxygen) promoted the degradation of 96.4% and 98.2% of anthocyanins after 48 and 96 h of fermentation, respectively. ESI-QToF-MS showed in positive-ion mode malvidin (molecular ion at m/z 331.0 showing [M – Cl]⁺ generating fragments at m/z 315.48), delphinidin (molecular ion at m/z 465.24 generating fragments at m/z 304.38, 303.88), and petunidin (molecular ion at m/z 479.38 generating fragments at m/z 317.30, 316.57) (Table 2).

On the other hand, nine phenolics compounds were identified in raw, cooked, and fermented beans (48 and 96 h). The positive-ion mode included naringenin (molecular ion m/z 273.00, fragments m/z 153.10, 147.12), ferulic acid (molecular ion m/z 177.14 lost H2O, fragments m/z 145.10, 117.58), gallic acid (molecular ion m/z 171.13, fragments m/z 153.12, 107.04, 79.08), rosmarinic acid (molecular ion m/z 383.18 showing [M + Na]⁺, fragments at m/z 221.13, 185.10, 163.12) and quercetin (molecular ion m/z 303.27, fragments m/z 229.14, 153.10). In the negative-ion mode, other phenolics were identified including ellagic acid (molecular ion at m/z 301.27 with fragments at m/z 284.02, 145.07), chlorogenic acid (molecular ion at m/z 353.34 with fragments at m/z 191.09, 190.06), rutin (molecular ion at m/z 609.08 with fragments at m/z 300.05, 301.06), and catechin (molecular ion at m/z 289.23 with fragments at m/z 288.09).
3.2 | In silico assays

Peptides sequences presented a negative theoretical affinity for α-amylase ranging from −0.3 to −8.3 kcal/mol (Table 3). Peptide SSVPW from fermented 96 h presented the lowest predicted binding energy. In the case of α-glucosidase, the predicted binding energies ranged from +0.1 to −5.5 kcal/mol, the peptide NPTPAGPVAPA from the raw sample presented the lowest binding for this enzyme. Binding energies for PLA2 ranged from −4.7 to −8.3 kcal/mol; the sequence SGGGF from fermented 96 h showed the lowest binding value. Predicted binding energies for MGL ranged from +3.6 to −9.8. The peptide TKPGGGAGP from fermented 96 h showed the lowest binding value. The PPARγ theoretical binding ranged from −0.3 to −7.8; the peptide SVGGGTA from fermented 96 h presented the highest inhibitory potential.

On the other hand, phenolic compounds from beans were good inhibitors of selected molecular markers (Table 4). Interaction with α-amylase catalytic site presented negative affinity values, −6.1 kcal/mol for rutin and −8.9 kcal/mol for quercetin. For α-glucosidase, the binding energies were −4.2 kcal/mol for ferulic acid and gallic acid and −6.1 kcal/mol for delphinidin. PLA2 inhibition values ranged from −5.4 kcal/mol (gallic acid) to −8.7 kcal/mol (rutin). The enzyme MGL inhibition ranged from −7.1 kcal/mol (gallic acid) to −10.1 kcal/mol (rutin) (Figure 5). In the case of PPARγ, the theoretical binding energies ranged from −5.8 kcal/mol (gallic acid) to −8.6 kcal/mol (naringenin).
3.3 | Biological potential

3.3.1 | α-Amylase and α-glucosidase inhibition

Protein hydrolysates generated from cooked black bean showed an IC$_{50}$ 5.23 mg soluble protein/ml for α-amylase, presenting significant differences compared with raw and SSF 48 h and 96 h samples (IC$_{50}$ 0.73, 0.598, and 0.57 mg soluble protein/ml, respectively) (Figure 6a). On the other hand, α-glucosidase (Figure 6b) presented lower IC$_{50}$ values in cooked beans and fermented 96 h (6.29 and 5.55 mg soluble protein/ml, respectively). Compared with raw bean and fermented 48 h (17.19 mg soluble protein/ml for raw bean and 13.55 mg protein/ml, respectively) ($p < 0.05$).

The α-amylase inhibition of phenolic compounds (Figure 6c) ranged from 0.104 mg to 0.178 mg GAE/ml with no significant differences among treatments. The inhibition of α-glucosidase using phenolic compounds (Figure 6d) showed that fermented 48 h and cooked bean presented the potential to block the enzyme with IC$_{50}$ values ranging from 0.353 and 0.361 mg GAE/ml, respectively, followed by 0.434 mg GAE/ml of raw black bean and 0.510 mg GAE/ml for fermented 96 h ($p < 0.05$).
Antioxidant capacity

Antioxidant capacity of protein hydrolysates associated with the radical ABTS (Figure 7a) ranged from 5.7 mg soluble protein/ml (raw back bean) to 27.14 mg soluble protein/ml (cooked bean) (p < 0.05). Protein hydrolysates from SSF showed similar antioxidant capacity, 10.68 and 11.30 mg soluble protein/ml for 48 and 96 h, respectively. In the inhibition of reactive oxygen species for the protein hydrolysates (Figure 7b), SSF showed the lowest IC50, with 0.630 and 1.16 mg soluble protein/ml for 96 and 48 h, respectively. Significant differences were observed when compared with raw samples IC50 3.45 mg soluble protein/ml and cooked samples IC50 9.01 mg soluble protein/ml.

Antioxidant capacity of phenolic compounds extracts for ABTS (Figure 7c) showed significant differences in fermented 96 h (IC50 0.719 mg GAE/ml) compared with raw, cooked, and fermented 48 h (IC50 0.322, 0.249, and 0.256 mg GAE/ml, respectively). In the case of inhibition of reactive oxygen species from phenolics compounds (Figure 7d), the antioxidant potential is maintained with no significant differences among treatments IC50 2.94, 2.96, and 3.78 μg GAE/ml for raw, cooked, and fermented 48 h treatments, respectively.

3.3.3 | Lipid accumulation

Cooked and fermented 96 h protein hydrolysates (0.5 mg/ml) presented lipid accumulation promoting effect (2.9% and 5.9% respectively). Contrary, in the case of raw and fermented 48 h...
treatments, lipid accumulation inhibition effect was observed at the same concentration (16.2% and 6.5%, respectively) (Figure 8a); 0.1 mg/ml of protein hydrolysates showed a lipid accumulation inhibitory effect ranging from 13.2% to 27.9% with no significant differences among treatments. Regarding phenolic compounds (Figure 8b), the concentrations tested showed a lipid accumulation effect ranging from 19.4% to 26.8% at 7.5 μg GAE/ml and 28.1% to 36.2% at 2.2 μg GAE/ml with no significant differences among treatments.

4 | DISCUSSION

4.1 | Characterization

4.1.1 | Protein characterization

The protein profile shows that GI digestion and SSF were not enough to completely hydrolyze the phaseolin band; however, it was partially hydrolyzed leading the appearance of bands around
This result contrast with reported by Mojica, Chen, and de Mejía (2015), where pepsin–pancreatin completely hydrolyzed precooked black bean proteins. The cooking process was not able to breakdown phaseolin; however, it facilitated its complete hydrolysis during the GI process. This could be due to a greater unfolding of the tertiary structure of proteins that allowed higher enzymatic cleavage.

The DH of cooked samples is similar to Noman et al. (2018); they found a relationship between temperature and protein DH. During SSF, *B. subtilis* hydrolyze peptide bonds (Dey

---

### Table 4: Molecular docking of identified phenolic compounds with selected molecular markers

| Phenolic Compound | Amylase kcal/mol | Glucosidase kcal/mol | PLA2 kcal/mol | MGL kcal/mol | PPAR kcal/mol |
|------------------|------------------|----------------------|--------------|--------------|--------------|
| Ferulic acid     | 6.5              | 4.2                  | 6.3          | 7.8          | 6.1          |
| Gallic acid      | 6.2              | 4.2                  | 5.4          | 7.1          | 5.8          |
| Rosmarinic acid  | 7.9              | 5.0                  | 7.6          | 9.7          | 7.8          |
| Naringenin       | 8.7              | 5.2                  | 7.9          | 9.8          | 8.6          |
| Quercetin        | 8.9              | 5.9                  | 7.4          | 9.7          | 8.3          |
| Malvidine        | 8.4              | 4.8                  | 7.7          | 8.9          | 7.2          |
| Delphinidin      | 8.5              | 6.1                  | 7.3          | 9.2          | 8.1          |
| Petunidin        | 8.5              | 6.0                  | 7.5          | 9.4          | 7.7          |
| Catechin         | 8.8              | 6.0                  | 7.6          | 9.1          | 7.9          |
| Ellagic acid     | 8.2              | 5.6                  | 7.5          | 8.8          | 8.4          |
| Chlorogenic acid | 8.1              | 5.3                  | 7.4          | 8.6          | 7.9          |
| Rutin            | 6.1              | 4.5                  | 8.7          | 10.1         | 7.5          |
| Pioglitazone     |                  | 8.1                  | 10.4         | 8.1          |
| Acarbose         | 7.7              | 5.0                  |              |              |              |

Note: Theoretical affinity (kcal/mol) of phenolics compounds from raw black bean, cooked black bean, and fermented black bean by 48 and 96h in the catalytic site of amylase, glucosidase, PLA2 (phospholipaseA2), MGL (monoacylglycerol lipase), and PPAR (peroxisome proliferatoractivated receptor gamma).
et al., 2016), showing similar DH for SSF and cooking treatments. After GI digestion with pepsin–pancreat-in, DH increased considerably, raw bean and fermented 48 h, showed similar values ($p > 0.05$). However, the process of cooking and fermented 96 h showed significant differences. This could be due to the higher rupture of peptide bonds associated with temperature and the microorganism action, leading to the exposure of amino groups for GI digestion (Joehnke et al., 2018). Also, Al-Ruwaih, Ahmed, Mulla, and Arfat (2019) reported an increment in the DH after hydrolysis of kidney bean protein isolates using alcalase.

4.1.2 | Polyphenols characterization

The content of total polyphenols, flavonoids, anthocyanins, and tannins of raw black bean correspond to data reported for Mojica, Meyer, et al. (2015) (Figure 4), where the largest fraction corresponds to tannins. The thermic process such as traditional cooking increased the number of total polyphenols; however, the increment was not statistically different ($p > 0.05$). Besides, Moreno-jim, Estrella, and Garcia (2014) reported a significant increase in phenolic compounds after thermal treatment. Furthermore, they found a similar decrease in anthocyanin content. This could be due to anthocyanin sensitivity to temperatures above 40°C, light, and oxygen. The reduction of total tannins during the thermal process corresponds to reported by Wang, Hatcher, Tyler, Toews, and Gawalko (2010) (78.6% of lost) compared to 80.0% reduction in this work. This could be attributed to high temperature, which hydrolyzes the polymeric structure of tannins and releases free phenolic compounds.

During SSF 48 and 96 h, Bacillus subtilis promote phenolic compounds released from the food matrix, mainly by the hydrolysis of tannins, improving its bioaccessibility and bioavailability (Valero-Cases,
Nuncio-Jáuregui, & Frutos, 2017). Also, synthesis of β-glucosidase hydrolyzes polysaccharides and disaccharides to monosaccharides (Chamoli, Kumar, Navani, & Verma, 2016). The conditions of SSF (light and oxygen) generated the degradation of 96.4% and 98.2% of anthocyanins after 48 and 96 h of fermentation, respectively.

Several research groups have reported similar phenolic compounds in common beans compared with this work. Furthermore, some phenolic compounds were identified among different samples including raw, cooked, and fermented beans, except for the catechin that was not present in cooked beans (Aguilera et al., 2016; Bhanja Dey, Chakraborty, Jain, Sharma, & Kuhad, 2016; Dueñas et al., 2016). Fermented beans present higher phenolic compounds content compared with samples coming from raw and cooked beans. Similar results were reported by Limón et al. (2015).

4.2 | In silico assays

Computational docking among peptide sequences and phenolic compounds with molecular markers related to obesity and T2DM including α-amylase, α-glucosidase, PLA2, MGL, and PPAR-γ showed similar theoretical binding energies compared with positive controls. Enzymes PLA2 and MGL are related to the metabolism of fatty acids, participating in lipid oxidation and generating free fatty acids, which participate in lipotoxicity. This effect is considered an important factor in promoting insulin resistance in T2DM (Luna-Vital et al., 2017).

The phenolic compounds presented higher affinity α-amylase, compared with α-glucosidase. These results correlate to biochemical assays, where the I_{C_{50}} was lower in α-amylase compared with α-glucosidase. Phenolic compounds interaction with MGL enzyme indicates that these molecules could decrease the effects of lipotoxicity, reducing the release of free fatty acids. On the other hand, these molecules were able to block PPAR-γ; its overexpression increases adipocyte maturation and inflammation factors that are related to obesity and T2DM (Huang, Huang, Hou, Chi, & Huang, 2014; Lee, Lee, Lefevre, & Kim, 2014; Luna-Vital, 2015). Peptides physicochemical properties and size are important parameters related to their biological potential. For instance, peptides with hydrophobic residues show higher antioxidant potential. Furthermore, low molecular weight peptides could be absorbed easily and present higher affinity and specificity for molecular targets. Also, peptides with hydrophobic and hydrophilic amino acid residues could have higher inhibitory activity for target enzymes due to competitive inhibition in the enzyme catalytic site (Luna, Mejia, & Mendoza, 2015; Moreno-Valdespino, Luna-Vital, Camacho-Ruiz, & Mojica, 2020; Teixeira et al., 2019).

4.3 | Biological potential

4.3.1 | α-Amylase and α-glucosidase inhibition

Protein hydrolysates generated by SSF and raw bean were significantly more effective compared with peptides from the cooked bean to block α-amylase. This may be due to higher hydrolysis in cooked samples generated at high temperatures. In a study, Betancur-Ancona, Sosa-Espinoza, Ruiz-Ruiz, Segura-Campos, and Chel-Guerrero (2014) found that DH affects the functional properties of proteins. On the other hand, the inhibition of α-glucosidase by cooked bean and fermented 96 h protein hydrolysates were effective (p < 0.05) blocking this enzyme. These results agree with Oseguera-toledo, Gonzalez, Mejia, and Amaya-llan (2015) for α-amylase; they reported higher inhibition in samples with low DH and higher inhibition of α-glucosidase on samples with high DH.

The inhibition α-amylase by phenolic compounds was constant among treatments; no significant differences were observed. Phenolic compounds maintained their activity despite the treatments and changes in phenolic compounds profile. These results are similar to reported by Tan, Chang, and Zhang (2017) in black soybean phenolic extract. For α-glucosidase inhibition, phenolic compounds from the cooked bean and fermented 48 h were more potent inhibitors. This could be due to changes in phenolic compounds associated with the metabolism of B. subtilis during SSF (Valero-Cases et al., 2017). Cooking generated changes such as degradation and formation of new compounds from the degradation reactions (Moreno-jim et al., 2014). In the study of Johnson and De Mejia (2016), they found that phenolic compounds from fermented berries were able to interact with markets of T2DM. They explain that possibly the effect was due to primary degradation products during fermentation such as protocatechuic acid. The treatments with the best affinity for the tested enzymes were the cooked black bean and fermented 48 h.

4.3.2 | Antioxidant capacity

Protein hydrolysates from SSF show higher antioxidant capacity compared with cooked bean to block ABTS radical. In the case of ROS, SSF showed a higher antioxidant capacity compared with raw and cooked bean. Similar improvements in antioxidant capacity during SSF were reported by Juan and Chou (2010). Results for raw black bean are comparable with reported by Mojica, Gonzalez de Mejia, et al. (2017). Phenolics compounds did not present significant differences in ROS inhibition among treatments. However, fermented 96 h phenolic extracts did not show ROS inhibition. Hwan, Eun, Sik, and Man (2019) reported an increase of antioxidant potential at longer fermentation time. Nevertheless, prolonged fermentation time promote phenolic compounds degradation due to the presence of oxygen, light and temperature (Peñarrieta, Tejeda, Mollinedo, Vila, & Bravo, 2014).

4.3.3 | Lipid accumulation

Protein hydrolysates at a concentration of 0.1 mg/ml presented reduction lipid accumulation. This result is comparable with Oseguera Toledo, Gonzalez de Mejia, Sivaguru, and Amaya-llan (2016); they
reported that pinto Durango alcalase hydrolysates at a concentration of 0.1 mg/ml produced a 28% of inhibition in lipid accumulation. However, at a concentration of 0.5 mg/ml, lipid accumulation was promoted. Therefore, at concentrations equal or lower than 0.1 mg/ml is probably to observe a positive effect on lipid metabolism, potentially by interaction with key enzymes or transcription factors associated with the differentiation of adipocytes, such as PPAR-γ.

Regarding phenolic compounds, they promoted lipid accumulation at the concentrations used. Luna-Vital et al. (2017) found a higher production of intracellular triglycerides by anthocyanins rich extract from purple corn pericarp in mature adipocytes. However, in the case of lipid accumulation during adipocyte differentiation, anthocyanins-rich extract from purple corn pericarp were able to inhibit lipid accumulation and adipocyte differentiation.

Molecular docking of phenolic compounds showed high affinity for PPAR-γ, which modulate adipocyte differentiation. However, this study was performed in mature adipocytes. Phenolic probably mimics the mechanism of action of TDZs such as pioglitazone increasing insulin sensitivity and then promoting glucose uptake and lipid accumulation (Luna-Vital et al., 2017).

5 | CONCLUSION

SSF is a promising black bean processing method for the generation of functional ingredients with the potential to modulate markers related to type 2 diabetes and obesity. This process could increase the antioxidant and biological potential of common beans. Solid-state fermented black bean flour rich bioactive components could be used in functional foods formulation. However, further in vivo assays are needed to validate solid-state fermented black bean flour biological potential. Legume functional ingredients could be in the prevention of non-communicable diseases.

ACKNOWLEDGMENT

This study was supported by CONACYT, Scientific Projects to Address National Problems 2016 Grant No. 2081.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

ETHICS STATEMENT

This manuscript does not contain any studies with human or animal subjects.

AUTHOR CONTRIBUTION

S. A. Flores-Medellín conducted the experiments and helped to design the study, data analysis, and manuscript writing. R. M. Camacho-Ruíz, C. Guizar-González, E. A. Rivera-Leon, and I. M. Llamas-Covarrubias were collaborators of the project who helped to design the study, supervised sample processing, and revised the manuscript. L. Mojica designed the study, provide guidance, manuscript review, and editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Luis Mojica https://orcid.org/0000-0001-6994-8711

REFERENCES

ADA. (2019). Standards of medical care in diabetes. Journal of Clinical and Applied Research and Education, 42, 92–96.

Aguilera, Y., Mojica, L., Rebollo-Hernanz, M., Berhow, M., De Mejía, E. G., & Martín-Cabrejas, M. A. (2016). Black bean coasts: New source of anthocyanins stabilized by β-cyclodextrin copigmentation in a sport beverage. Food Chemistry, 212, 561–570. https://doi.org/10.1016/j.foodchem.2016.06.022

Al-Ruwaiti, N., Ahmed, J., Mulla, M. F., & Arfat, Y. A. (2019). High-pressure assisted enzymatic proteolysis of kidney beans protein isolates and characterization of hydrolysates by functional, structural, rheological and antioxidant properties. LWT, 100, 231–236. https://doi.org/10.1016/j.lwt.2018.10.074

Azzini, E., Giacometti, J., & Russo, G. L. (2017). Antibesity effects of anthocyanins in preclinical and clinical studies. Oxidative Medicine and Cellular Longevity, 2017(11), 1–11. https://doi.org/10.1155/2017/2740364

Betancur-Ancona, D., Sosa-Espinosa, T., Ruiz-Ruiz, J., Segura-Campos, M., & Chel-Guerrero, L. (2014). Enzymatic hydrolysis of hard-to-cook bean (Phaseolus vulgaris L) protein concentrates and its effects on biological and functional properties. International Journal of Food Science and Technology, 49(1), 2–8. https://doi.org/10.1111/ijfs.12267

Bhanja Dey, T., Chakraborty, S., Jain, K. K., Sharma, A., & Kuhad, R. C. (2016). Antioxidant phenolics and their microbial production by submerged and solid state fermentation process: A review. Trends in Food Science and Technology, 53, 60–74. https://doi.org/10.1016/j.tifs.2016.04.007

Chamoli, S., Kumar, P., Navani, N. K., & Verma, A. K. (2016). Secretory expression, characterization and docking study of glucose-tolerant β-glucosidase from B. subtilis. International Journal of Biological Macromolecules, 85, 425–433. https://doi.org/10.1016/j.ijbiomac.2016.01.001

Chavez-servia, J. L., Aquino-bolanos, E. N., Garcia-diaz, Y. D., & Chavez-servia, J. L. (2016). Anthocyanin, polyphenol, and flavonoid contents and antioxidant activity in Mexican common bean (Phaseolus vulgaris L.) landraces. Emirates Journal of Food and Agriculture, 28(8), 581. https://doi.org/10.9755/efja.2016-02-147

Chen, H., Wortmann, A., Zhang, W., & Zenobi, R. (2007). Rapid in vivo fingerprinting of nonvolatile compounds in breath by extractive electrospray ionization quadrupole time-of-flight mass spectrometry. Angewandte Chemie, International Edition, 46(4), 580–583. https://doi.org/10.1002/anie.200602942

Dey, T. B., Chakraborty, S., Jain, K. K., Sharma, A., & Kuhad, R. C. (2016). Trends in food science & technology antioxidant phenolics and their microbial production by submerged and solid state fermentation process: A review. Trends in Food Science & Technology, 53, 60–74. https://doi.org/10.1016/j.tifs.2016.04.007

Dueñas, M., Sarmento, T., Aguilera, Y., Benitez, V., Mollá, E., Esteban, R. M., & Martín-Cabrejas, M. A. (2016). Impact of cooking and germination on phenolic composition and dietary fibre fractions in dark beans (Phaseolus vulgaris L.) and lentils (Lens culinaris L.). LWT - Food Science and Technology, 66, 72–78. https://doi.org/10.1016/j.lwt.2015.10.025

Gadde, K. M., Martin, C. K., Berthoud, H. R., & Heymsfield, S. B. (2018). Obesity: Pathophysiology and management. Journal of the American
Peñarrieta, J., Tejeda, L., Mollinedo, P., Vila, J., & Bravo, J. (2014). Comuestos fenólicos y su presencia en alimentos. *Bolivian Journal of Chemistry, 31*(2), 68–81. https://doi.org/10.1007/s00394-008-2002-2

Pereira, S. S., & Alvarez-Leite, J. I. (2014). Low-grade inflammation, obesity, and diabetes. *Current Obesity Reports, 3*(4), 422–431. https://doi.org/10.1007/s13679-014-0124-9

San Pablo-Osorio, B., Mojica, L., & Urías-Silvas, J. E. (2019). Chía seed (Salvia hispanica L.) pepsin hydrolysates inhibit angiotensin-converting enzyme by interacting with its catalytic site. *Journal of Food Science, 84*(5), 1170–1179. https://doi.org/10.1111/1750-3841.14503

Sánchez, Y. G., Cruz-martín, M., Sánchez-garcía, C., & Leiva-mora, M. (2016). Contenido de fenoles totales en la testa de las semillas de tres cultivares de Phaseolus vulgaris L. *Biotecnología Vegetal, 16*(2), 125–128. eISSN 2074–8647

Tan, Y., Chang, S. K. C., & Zhang, Y. (2017). Comparison of α-amylase, α-glucosidase and lipase inhibitory activity of the phenolic substances in two black legumes of different genera. *Food Chemistry, 214*(July 2016), 259–268. https://doi.org/10.1016/j.foodchem.2016.06.100

Teixeira, R. I., Goulart, J. S., Corrêa, R. J., Garden, S. J., Ferreira, S. B., Netto-Ferreira, J. C., ... De Lucas, N. C. (2019). A photochemical and theoretical study of the triplet reactivity of furano- and pyrano-1,4-naphthoquinones towards tyrosine and tryptophan derivatives. *RSC Advances, 9*(24), 13386–13397. https://doi.org/10.1039/c9ra01939a

Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *Journal of Computational Chemistry, 31*, 455–461. https://doi.org/10.1002/jcc.21334

Valero-Cases, E., Nuncio-Jauregui, N., & Frutos, M. J. (2017). Influence of fermentation with different lactic acid bacteria and in vitro digestion on the biotransformation of phenolic compounds in fermented pomegranate juices. *Journal of Agricultural and Food Chemistry, 65*(31), 6488–6496. https://doi.org/10.1021/acs.jafc.6b04854

Wang, N., Hatcher, D. W., Tyler, R. T., Toews, R., & Gawalko, E. J. (2010). Effect of cooking on the composition of beans (Phaseolus vulgaris L.) and chickpeas (Cicer arietinum L.) q. *Food Research International, 43*(2), 589–594. https://doi.org/10.1016/j.foodres.2009.07.012

Zebisch, K., Voigt, V., Wabitsch, M., & Brandsch, M. (2012). Protocol for effective differentiation of 3T3-L1 cells to adipocytes. *Analytical Biochemistry, 425*(1), 882013:90. https://doi.org/10.1016/j.ab.2012.03.005

How to cite this article: Flores-Medellín SA, Camacho-Ruiz RM, Guízar-González C, Rivera-León EA, Llamas-Covarrubias IM, Mojica L. Protein hydrolysates and phenolic compounds from fermented black beans inhibit markers related to obesity and type-2 diabetes. *Legume Science*. 2021;3:e64. https://doi.org/10.1002/leg3.64