

Cookies elaborated with oat and common bean flours improved serum markers in diabetic rats

Iza F Pérez-Ramírez,^a Laura J Becerril-Ocampo,^a Rosalía Reynoso-Camacho,^a Mayra D Herrera,^b S Horacio Guzmán-Maldonado^c and Raquel K Cruz-Bravo^{b*} 

Abstract

BACKGROUND: Common beans have been associated with anti-diabetic effects, due to its high content of bioactive compounds. Nevertheless, its consumption has decreased worldwide. Therefore, there is an increasing interest in the development of novel functional foods elaborated with common beans. The aim of this study was to evaluate the anti-diabetic effect of oat–bean flour cookies, and to analyze its bioactive composition, using commercial oat–wheat cookies for comparative purposes.

RESULTS: Oat-bean cookies (1.2 g kg⁻¹) slightly decreased serum glucose levels (~1.1-fold) and increased insulin levels (~1.2-fold) in diabetic rats, reducing the hyperglycemic peak in healthy rats (~1.1-fold). Oat–bean cookies (0.8 and 1.2 g kg⁻¹) exerted a greater hypolipidemic effect than commercial oat–wheat cookies (1.2 g kg⁻¹), as observed in decreased serum triglycerides and low-density lipoprotein cholesterol. Furthermore, the supplementation with 1.2 g kg⁻¹ oat–bean cookies decreased atherogenic index and serum C-reactive protein levels, suggesting their cardioprotective potential. The beneficial effect of oat–bean cookies was associated with their high content of dietary fiber and galacto oligosaccharides, as well as chlorogenic acid, rutin, protocatechuic acid, β -sitosterol and soyasaponins.

CONCLUSION: These results suggest that common beans can be used as functional ingredients for the elaboration of cookies with anti-diabetic effects.

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Keywords: healthy snacks; *Phaseolus vulgaris* L; *Avena sativa*; phytochemicals; dietary fiber; diabetes

INTRODUCTION

Diabetes is considered a global emergency, since about 415 million adults are estimated to have diabetes, whereas 318 million adults show impaired glucose tolerance and thus have a high risk of developing diabetes in the future. Moreover, diabetes represents 14.5% of global mortality in people aged between 20 and 79 years. Diabetes has a considerable economic impact due to health services regarding diabetes control and long-term diabetes-related complications, as well as productive loss.¹

Diabetes is characterized by high blood glucose levels due to a decreased insulin secretion or activity, leading to alterations in plasmatic lipids, as well as the development of vascular complications, such as diabetic nephropathy and hepatic steatosis.² Dietary recommendations for diabetes primary treatment include the consumption of functional foods, including common beans (*Phaseolus vulgaris* L.), a worldwide consumed legume.³

The consumption of legumes (>3 servings per week) has been associated with a decreased risk of diabetes (20–35%) in middle-aged women.⁴ Moreover, it has been reported that beans attenuate the glycemic response after rice consumption in adults with type 2 diabetes.^{4,5} Similarly, the consumption of canned beans showed lower postprandial glucose levels as compared to bread intake in type 2 diabetics.⁶ Furthermore, we have previously reported that supplementation with cooked common beans decreases hyperglycemia and dyslipidemia in diabetic

rats.^{7,8} These beneficial effects may be related to their high content of dietary fiber, phenolic acids, flavonoids, phytosterols and saponins.^{9,10}

The overall consumption of beans has decreased in several countries, including Mexico, reducing from 13.2 kg per capita in 1995 to 8.4 in 2016 according to Trust Funds for Rural Development (FIRA). This situation has been related to changes in consumption preferences. Hence several bean-based products have been developed, such as tortillas and spaghetti pasta, which showed a high content of protein, dietary fiber and polyphenols.^{11,12}

In addition to common beans, oats (*Avena sativa*) have been used as ingredients for the development of food products with health benefits. This cereal is a rich source of soluble fiber, which

* Correspondence to: RK Cruz-Bravo, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Zacatecas, Calera 98500, México. E-mail: raquelcb81@gmail.com

a Facultad de Química, Universidad Autónoma de Querétaro, Querétaro, México

b Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Zacatecas, Calera, México

c Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Bajío, Celaya, México

reduces glucose levels and improves insulin response, and exerts hypolipidemic and anti-obesogenic effects.¹³ The aim of this study was to evaluate the anti-diabetic effect of cookies elaborated with oat and common bean flours and to determine their dietary fiber and phytochemical composition.

EXPERIMENTAL

Oat-bean flour cookie elaboration

Pinto Saltillo beans were acquired from plants grown at INIFAP-Zacatecas under partial irrigation conditions. Seeds were placed in an oven (Fisher Scientific Isotemp Oven, 66G) at 60 °C until dryness, then ground in a stone mill. Bean flour was stored at 4 °C protected from light. Cookies were elaborated with 27.60% oat flour, 24.80% common bean flour, 2.80% whole-wheat flour, 20.70% no-salt no-*trans*-fat margarine, 13.80% water, 6.06% nut, 2.89% vanilla essence, 0.30% baking powder, 0.2% industrial stevia powder, 0.2% cinnamon powder and 0.2% salt. Cookies were baked at 180 °C for 40 min (Bosch, P4 Safety Cook). After cooling, oat-bean cookies were stored hermetically in a cool and dry room for further analysis. Commercial oat-wheat cookies with no added sugar were used for comparative purposes.

Total dietary fiber (TDF) content

Total, soluble and insoluble dietary fiber (TDF, SDF and IDF, respectively) were determined using the enzymatic-gravimetric method as described by Prosky *et al.*¹⁴

Resistant starch (RS) content

RS content was determined as described by Saura-Calixto.¹⁵ Briefly, 100 mg IDF was mixed and continuously shaken with 6 mL of 2 mol L⁻¹ KOH for 30 min at room temperature. Then, 3 mL acetate buffer (0.4 mol L⁻¹, pH 4.75) and 5 mL of 2 mol L⁻¹ HCl were added, adjusting pH to 4.75 with 2 mol L⁻¹ HCl. Afterwards, 60 µL amyloglucosidase was added. The solution was mixed and incubated for 30 min at 60 °C with continuous stirring. Then, samples were centrifuged for 15 min at 3000 × *g* and supernatants were collected. Pellets were resuspended in 10 mL distilled water and centrifuged for 15 min at 3000 × *g*. Both supernatants were combined in a 50 mL flask and adjusted to a final volume of 50 mL with distilled water. Total glucose was quantified using the glucose oxidase-peroxidase kit (GLUC-PAP, GL2614 from RANDOX). Absorbances were measured at 500 nm against a blank reagent. The RS was calculated as glucose (mg) × 0.9.

Oligosaccharide quantification

Oligosaccharides were extracted from oat-wheat and oat-bean cookies following the procedure described by Brenes *et al.*¹⁶ Samples (10 g) were homogenized with aqueous ethanol (100 mL, 80%, v/v) and placed in a Soxhlet instrument at 80 °C for 60 min. The ethanol extracts were recovered and concentrated under vacuum, and the water phase was lyophilized. Oligosaccharides of lyophilized samples were quantified as described by Muzquiz *et al.*¹⁷ Briefly, samples (7 mg) were dissolved in deionized water (1 mL), filtered and subjected to analysis by high-performance liquid chromatography (HPLC). Samples (20 µL) were injected into an Agilent HPLC system (model HP-1100, Agilent Technologies, Inc., Santa Clara, CA, USA) with a refractive index detector (RID, 61362A) and fitted with a Zorbax NH2 precolumn (4.6 × 12.6 mm, 5 µm) and Zorbax column (250 × 4.6 mm). Acetonitrile-water (65:35) was used as mobile phase at 1 mL min⁻¹. Column and detector

temperatures were maintained at 25 °C. Standard curves were determined using raffinose, stachyose and verbascose standards.

Total phytochemical content and HPLC-diode array detection – mass selective detection profile

Total phenolics were estimated by the Folin-Ciocalteu assay,¹⁸ and results were expressed as milligrams of gallic acid equivalents per gram (mg GAE g⁻¹). Total flavonoids were determined using the method previously described by Chang *et al.*,¹⁹ and results were expressed as milligrams of catechin equivalents per gram (mg CE g⁻¹). Total saponins were estimated using the assay described by Hiai *et al.*,²⁰ and results were expressed as milligrams of soya-aponin I equivalents per gram (mg SSE g⁻¹). Total phytosterols were quantified using the method described by Do Prado *et al.*,²¹ and results were expressed as milligrams of β-sitosterol equivalents per gram (mg SE g⁻¹).

The identification and quantification of phytochemicals were assessed in an Agilent 1200 high-performance liquid chromatograph connected to a diode array detector and a single-quadrupole mass spectrometer, equipped with an electrospray ionization (ESI) interphase, using a Zorbax ODS-18 (15 × 4.6, 5 µm) column at 40 °C. The mass spectrometer was operated in the negative ion mode, using the following conditions: capillary voltage, 4000 V; nebulizer pressure, 40 psi; drying gas flow rate, 10 L min⁻¹; gas temperature, 300 °C; skimmer voltage, 50 V; octupole, 150 V; and fragment voltage, 130 V. Mass spectra were recorded across the range *m/z* 50–1400. Three different systems were used for the identification of phytochemical compounds.⁸

System I (phenolic acids and flavonoids)

Ground cookies (50 mg) were extracted twice with 500 µL acetone-water 70:30 (v/v), homogenized at 24 000 rpm and centrifuged at 10 000 × *g* at 4 °C for 5 min. Both supernatants were combined, evaporated to dryness and dissolved in 100 µL mobile phase. The binary solvent system (flow rate of 0.8 mL min⁻¹) consisted of solvent A (water containing 1% formic acid) and solvent B (acetonitrile) under gradient conditions: 95/5 (A/B) from 0 to 20 min, 80/20 from 20 to 25 min and 60/40 from 25 to 30 min. Absorbances were measured at 260, 280 and 320 nm. Quantification was carried out using standards of phenolic acids (chlorogenic acid, gallic acid, *p*-hydroxybenzoic acid, caffeic acid, protocatechuic acid, *p*-coumaric acid, rosmarinic acid, ferulic acid, sinapic acid and ellagic acid) and flavonoids (epicatechin, catechin, gallic acid, gallic acid, epigallocatechin gallate, quercetin, hesperidin, and rutin). Results are expressed as ng g⁻¹.

System II (saponins)

Ground cookies (50 mg) were extracted twice with 500 µL methanol-water 80:20 (v/v) as previously described. The binary solvent system (flow rate of 0.4 mL min⁻¹) consisted of solvent A (acetonitrile containing 0.1% formic acid) and solvent B (water containing 0.1% formic acid) under gradient conditions: 75/25 A/B from 0 to 3 min, 50/50 from 3 to 20 min and 20/80 from 20 to 30 min. Absorbances were measured at 205 nm. Quantification was carried out using soya-aponin I as standard, and results are expressed as µg SSE g⁻¹.

System III (phytosterols)

Ground cookies (50 mg) were extracted twice with 500 µL *n*-hexane as previously described. The binary solvent system

(flow rate of 0.8 mL min⁻¹) consisted of solvent A (methanol) and solvent B (water containing 1% acetonitrile) under gradient conditions: 85/15 A/B from 0 to 15 min and 100/0 from 15 to 30 min. Absorbances were measured at 205 nm. Quantification was carried out using β -sitosterol as standard, and results are expressed as $\mu\text{g SE g}^{-1}$.

Experimental animals

Male Wistar rats (250 \pm 20 g) were acquired from the Universidad Nacional Autónoma de México (Campus Querétaro, México), and were maintained at 24 \pm 1 °C under a 12/12 h light/dark cycle. Experiments on animals were performed by following the guidelines of the National Research Council, approved by the Bioethics Committee of the Universidad Autónoma de Querétaro, México.

Acute fasting glycemic test

Blood glucose levels were measured after the administration of cookies in fasting healthy rats ($N=20$). Ground cookies were dissolved in water. Then, oat–bean cookies (0.4, 0.8 and 1.2 g kg⁻¹ body weight) and commercial oat–wheat cookies (1.2 g kg⁻¹ body weight) were administered intragastrically to the corresponding group, while the negative control was given distilled water. These doses are equivalent to the daily consumption of three (30 g), six (60 g) and nine (90 g) oat–bean cookies. Afterwards, glucose levels were measured at 0, 30, 60, 90 and 120 min using blood obtained from the tail vein with a reflective glucometer (Accucheck, Roche Diagnostics, Mannheim, Germany). The area under the curve (AUC) was calculated using the trapezoidal function.²²

Acute oral glucose tolerance test

Fasting healthy rats ($N=20$) were used for this experiment. Cookies were administered intragastrically as previously described, followed by the administration of a glucose load (2 g kg⁻¹). Blood glucose levels were measured as previously described and AUC values were estimated.

Diabetes induction and experimental design

Animals were divided randomly into six groups of seven animals each. The healthy control group was fed a standard diet (Rodent Lab chow 5001). The diabetic control and the four treatment groups were fed a high-fat diet (HFD) (standard diet added with 28% fat). Diets were administered *ad libitum* for 2 months. Afterwards, diabetes was induced in animals fed with HFD through an intraperitoneal injection of streptozotocin (STZ; 35 mg kg⁻¹ body weight in 0.1 mol L⁻¹ citrate buffer at pH 4.5). After 1 week of induction, blood samples were withdrawn from the tail vein and glucose concentration was measured with a reflective glucometer. Rats with a fasting glucose ≥ 200 mg dL⁻¹ were considered diabetic.

Diabetic animals were divided into five groups: the diabetic control was fed with HFD, whereas the other four diabetic groups were fed with HFD supplemented with oat–bean cookies (0.4, 0.8 or 1.2 g kg⁻¹ body weight) or commercial oat–wheat cookies (1.2 g kg⁻¹ body weight). Diets were administered *ad libitum* for 6 weeks; afterwards, rats were anesthetized and blood was collected by cardiac puncture.

Quantification of serum biochemical parameters

Blood samples were centrifuged at 3000 $\times g$ at 4 °C for 10 min, and then serum glucose, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL) and high-density lipoprotein

Table 1. Dietary fiber, resistant starch and oligosaccharide content of oat–bean flour cookies

Component	Oat–bean cookie	Commercial oat–wheat cookie
Total dietary fiber	15.75 \pm 1.07a	ND
Soluble fiber	10.2 \pm 0.63a	ND
Insoluble fiber	5.53 \pm 0.43a	ND
Resistant starch	8.72 \pm 0.42a	ND
Raffinose	5.68 \pm 0.35a	1.68 \pm 0.06b
Stachyose	1.76 \pm 0.07a	0.76 \pm 0.01b
Verbascode	ND	ND

Data are presented as the mean \pm standard error. Different letters within each row indicate significant ($P < 0.05$) difference by *t*-test. ND, not detected.

cholesterol (HDL) were measured using enzymatic-colorimetric kits (Spinreact, Sant Esteves de Bas, Spain), whereas C-reactive protein was determined using enzyme-linked immunoassay kits (ALPCO, NH, USA). Atherogenic index was estimated as follows: atherogenic index = total cholesterol (mg dL⁻¹)/HDL (mg dL⁻¹).²³

Determination of serum hepatic dysfunction markers

Serum alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined using enzymatic–colorimetric kits (Spinreact).

Statistical analysis

All data are expressed as mean values \pm standard error. Statistical significance was determined by ANOVA followed by Tukey's multiple means comparison test ($P < 0.05$). All the statistical analyses were carried out with JMP software (v11.0, SAS Institute).

RESULTS

Dietary fiber and oligosaccharides

Oat–bean cookies showed a significantly higher content of dietary fiber than the commercial oat–wheat cookie (Table 1). Under the experimental conditions used in this study, TDF, IDF, SDF and RS were not detected in the commercial oat–wheat cookies. Regarding galacto-oligosaccharide content, oat–bean cookies showed a larger amount of raffinose and stachyose as compared to the commercial oat–wheat cookies (3.3- and 2.3-fold, respectively), whereas verbascode was not detected in either cookie.

Phytochemical composition of oat–bean flour cookies

The oat–bean cookies presented the highest content of total polyphenols and flavonoids as compared to commercial oat–wheat cookies (1.27- and 1.78-fold, respectively) (Table 2). Regarding the polyphenol profile, ten phenolic acids and seven flavonoids were identified. Both cookies presented chlorogenic acid as the major compound, followed by catechin and epicatechin. Additionally, oat–bean cookies presented a high content of rutin, which was not detected in commercial oat–wheat cookies.

Regarding total saponins, oat–bean cookies presented the highest content as compared to commercial oat–wheat cookies (1.85-fold). Additionally, eight steroidal saponins were identified, and soyasaponin Ba (V) was the major compound of oat–bean cookies, followed by soyasaponin β g. On the other hand, total phytoosterols were detected only in oat–bean cookies. Nevertheless,

Table 2. Polyphenols saponin and phytosterol profile of oat–bean flour cookies

Phytochemical compound	Retention time (min)	<i>m/z</i>	Oat–bean cookie	Commercial oat–wheat cookie
Total polyphenols (mg GAE g ⁻¹)	–	–	8.58 ± 0.40a	6.74 ± 0.43b
Total flavonoids (mg CE g ⁻¹)	–	–	0.41 ± 0.02a	0.23 ± 0.01b
Total saponins (μg SSE g ⁻¹)	–	–	185.09 ± 18.15a	99.92 ± 8.54b
Total phytosterols (μg SE g ⁻¹)	–	–	171.57 ± 47.21a	ND
Phenolic acids (μg g ⁻¹)				
Chlorogenic acid	1.7	353	1528.41 ± 6.55b	1767.33 ± 5.75a
Gallic acid	3.7	169	7.62 ± 0.19a	8.50 ± 0.56a
<i>p</i> -Hydroxybenzoic acid	4.3	137	43.58 ± 0.82a	25.37 ± 0.11b
Caffeic acid	13.7	179	24.63 ± 0.39a	ND
Protocatechuic acid	14.3	153	69.98 ± 0.43a	2.26 ± 0.03b
Coumaric acid	16.1	163	0.82 ± 0.01b	1.69 ± 0.06a
Rosmarinic acid	16.6	359	ND	3.80 ± 0.17a
Ferulic acid	18.9	193	5.22 ± 0.14a	ND
Sinapic acid	21.2	223	1.30 ± 0.09a	ND
Ellagic acid	22.2	301	14.18 ± 0.52a	ND
Flavonoid profile (μg g ⁻¹)				
Epicatechin	2.3	289	192.09 ± 3.79b	235.18 ± 1.44a
Catechin	2.7	289	100.32 ± 3.30b	190.62 ± 1.65a
Gallocatechin gallate	14.8	457	0.82 ± 0.01a	ND
Epigallocatechin gallate	15.9	457	2.02 ± 0.03b	2.86 ± 0.10a
Quercetin	18.1	609	38.71 ± 0.28a	27.22 ± 0.45b
Hesperidin	23.5	609	10.97 ± 0.16a	ND
Rutin	27.8	609	107.90 ± 1.57a	ND
Saponin profile (μg SSE g ⁻¹)				
Phaseoside I	8.4	1252	14.15 ± 0.08a	10.43 ± 0.08b
Soysaponin Ba (V)	12.6	958	36.63 ± 0.31a	10.01 ± 0.20b
Soysaponin Bb (I)	15.9	942	10.50 ± 0.13a	9.32 ± 0.15a
Soysaponin Af	17.2	1274	9.15 ± 0.04a	6.61 ± 0.08b
Soysaponin Bd	19.5	956	8.51 ± 0.23a	6.20 ± 0.05b
Soysaponin γg	22.4	922	11.23 ± 0.19a	9.19 ± 0.09b
Soysaponin βg	24.3	1068	15.50 ± 0.23a	10.66 ± 0.08b
Soysaponin αg	24.7	1084	10.88 ± 0.17a	7.41 ± 0.06b
Phytosterol profile (μg SE g ⁻¹)				
Brassicasterol	2.1	397	3.91 ± 0.06a	3.33 ± 0.07b
Ergosterol	2.3	395	2.70 ± 0.06a	1.28 ± 0.06b
Fucosterol	2.7	411	4.07 ± 0.08a	2.12 ± 0.04b
Δ5-Avenasterol	3.8	411	10.72 ± 0.15a	7.31 ± 0.03b
Δ7-Stigmasterol	6.3	411	7.95 ± 0.09a	4.86 ± 0.03b
β-Sitosterol	7.3	413	12.65 ± 0.22a	1.40 ± 0.03b
β-Campesterol	8.7	399	23.55 ± 0.09a	13.77 ± 0.71b
Δ7-Avenasterol	16.9	411	6.90 ± 0.11a	1.80 ± 0.01b
Stigmastanol	17.1	415	4.88 ± 0.07b	5.79 ± 0.06a
Sitosteryl-3-β-glucopyranoside	19.3	575	18.41 ± 0.12a	12.13 ± 0.09b
Campesteryl-3-β-glucopyranoside	20.4	561	3.94 ± 0.04a	0.31 ± 0.03b
Stigmasteryl-3-β-glucopyranoside	20.7	573	3.56 ± 0.07a	1.73 ± 0.04b

Data are presented as the mean ± standard deviation (*n* = 3). GAE, gallic acid equivalents; CE, catechin equivalents; SSE, soysaponin I equivalents; SE, β-sitosterol equivalents. Different letters within each row indicate significant (*P* < 0.05) difference by *t*-test.

nine free phytosterols and three glucoside phytosterols were identified in both cookies. β-Campesterol was the major compound of both cookies, which was found in greater amounts in oat–bean cookies, followed by sitosteryl-β-D-glucopyranoside, β-sitosterol and Δ5-avenasterol.

Effect of oat–bean flour cookies on acute fasting glucose levels in healthy rats

The effect of oat–bean cookies (0.4, 0.8 and 1.2 g kg⁻¹) and commercial oat–wheat cookies (1.2 g kg⁻¹) was evaluated on postprandial glucose levels after their intragastric administration

(Fig. 1). The administration of all cookies significantly (*P* < 0.05) increased the hyperglycemic peak as compared to the negative control group (1.16–1.40-fold) (Fig. 1A).

Rats treated with commercial oat–wheat cookies presented the highest blood glucose levels (160 mg dL⁻¹), followed by the three doses of oat–bean cookies (132.5–136.5 mg dL⁻¹) (Fig. 1A). After 120 min, all animals treated with oat–bean cookies presented basal glucose levels (98.5–99.5 mg dL⁻¹), whereas rats treated with commercial oat–wheat cookies presented slightly higher glucose levels (112 mg dL⁻¹). Accordingly, animals treated with oat–bean cookies presented similar AUC values as compared

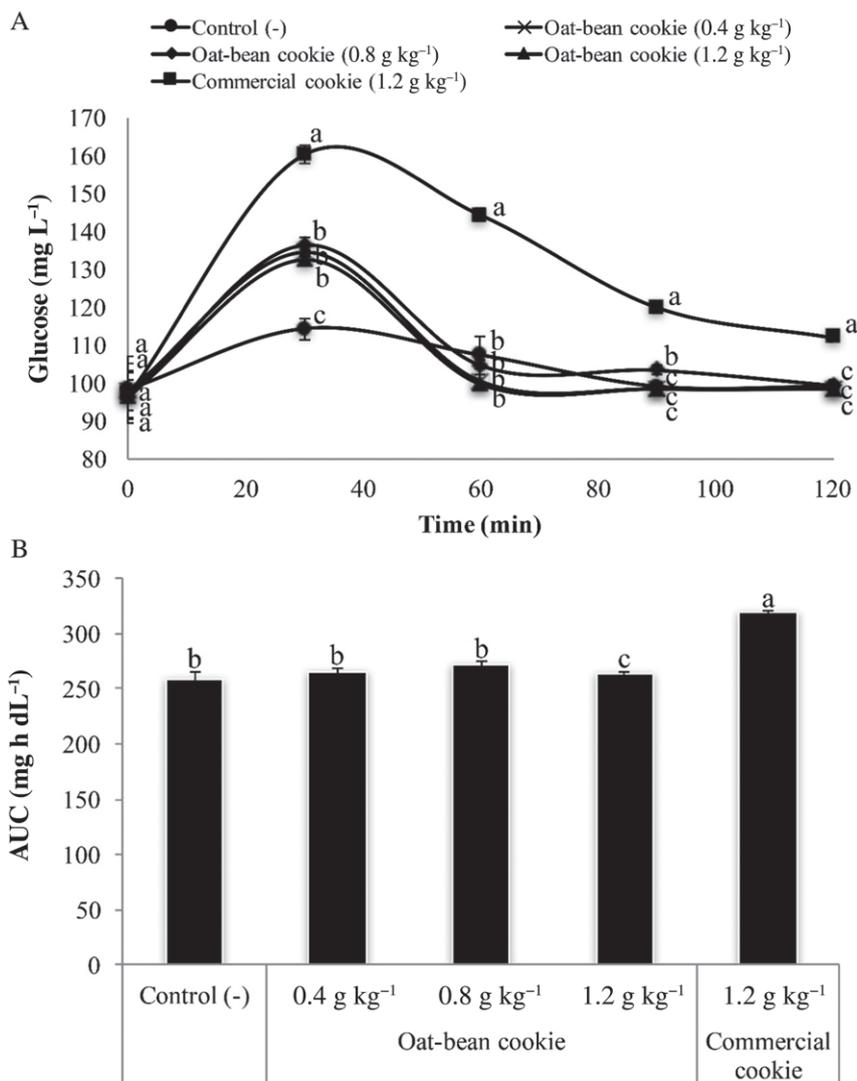


Figure 1. Effect of oat-bean flour cookies on postprandial glucose curve (A) and AUC values (B) in healthy rats. Data are presented as means and error bars indicate standard error ($n = 7$). Different letters indicate significant ($P < 0.05$) difference by Tukey's test. AUC, area under the curve.

to the negative control group, whereas the administration of commercial oat-wheat cookies increased AUC values (1.23-fold) (Fig. 1B).

Effect of oat-bean flour cookies on acute oral glucose intolerance in healthy rats

An oral glucose tolerance test was performed in healthy rats, which were administered cookies as previously described, followed by a glucose load (Fig. 2). The hyperglycemic peak was reached at 30 min by all animals, and the group treated with commercial oat-wheat cookies showed the highest glucose levels (205 mg dL⁻¹), presenting significantly ($P < 0.05$) increased values as compared to the negative control group (1.22-fold) (Fig. 2A). On the other hand, the administration of 0.8 and 1.2 g kg⁻¹ oat-bean cookies significantly ($P < 0.05$) decreased the hyperglycemic peak as compared to the negative control group (1.11- and 1.14-fold, respectively), whereas no difference was observed between the lowest dose of oat-bean cookies (0.4 g kg⁻¹) and the negative control group.

After 120 min, the negative control group presented basal glucose levels; however, all animals treated with cookies presented

high glucose levels (135–157 mg dL⁻¹). Regarding AUC values, animals treated with the lowest and highest dose of oat-bean cookies (0.4 and 1.2 g kg⁻¹, respectively) presented similar values as compared to the negative control group (Fig. 2B); whereas animals supplemented with the middle dose (0.8 g kg⁻¹) presented slightly higher AUC values as compared to the negative control group (1.67-fold), which may be related to the increased glucose levels at 120 min. On the other hand, animals treated with commercial oat-wheat cookies presented the highest AUC values (1.13-fold).

Effect of oat-bean flour cookies on serum glucose and insulin in diabetic rats

The diabetic control group presented increased serum glucose levels (2.4-fold) and decreased serum insulin levels (1.4-fold) as compared to the healthy control group (Fig. 3A and Table 3). No significant difference was observed between the cookie-treated groups and the diabetic control group. However, the administration of 0.8 and 1.2 g kg⁻¹ oat-bean cookies slightly decreased serum glucose levels as compared to the diabetic control group (1.08- and 1.13-fold, respectively); whereas the administration of 0.4 g kg⁻¹ oat-bean cookies and 1.2 g kg⁻¹ commercial

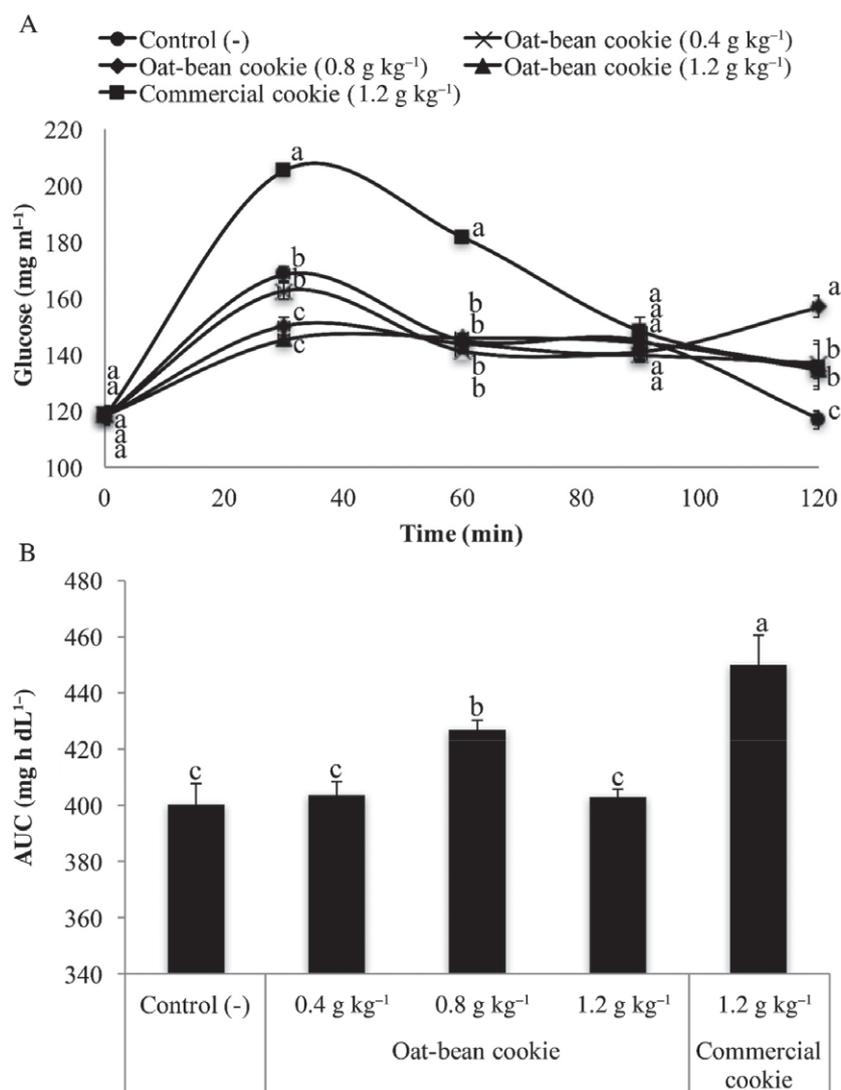


Figure 2. Effect of oat–bean flour cookies on glucose intolerance curve (A) and AUC values (B) in healthy rats. Data are presented as means and error bars indicate standard error ($n = 7$). Different letters indicate significant ($P < 0.05$) difference by Tukey's test. AUC, area under the curve.

oat–wheat cookies slightly increased serum glucose levels as compared to the diabetic control group (1.02–1.10-fold) (Fig. 3A). On the other hand, all treatments significantly ($P < 0.05$) increased serum insulin levels as compared to the diabetic group (1.12–1.25-fold), and the highest dose of oat–bean cookies (1.2 g kg⁻¹) exerted the greatest effect (Fig. 3B).

Effect of oat–bean flour cookies on serum lipid profile, atherogenic index and C-reactive protein in diabetic rats

The diabetic control group presented increased serum triglycerides (1.82-fold), total cholesterol (1.58-fold), and LDL (1.54-fold) values as compared to the healthy control group, as well as decreased serum HDL values (1.42-fold), which was reflected in an increased atherogenic index (2.6-fold). Furthermore, the diabetic control group presented a significant ($P < 0.05$) increase in serum C-reactive protein levels as compared to the healthy control group (2.4-fold) (Table 3).

The administration of 0.8 and 1.2 g kg⁻¹ oat–bean cookies significantly ($P < 0.05$) decreased serum triglycerides (1.20- and 1.28-fold, respectively), total cholesterol (1.18- and 1.27-fold, respectively) and LDL (1.28- and 1.31-fold, respectively) values as

compared to the diabetic control group, and increased HDL levels (1.52- and 1.74-fold, respectively). On the other hand, the lowest dose of oat–bean cookies (0.4 g kg⁻¹) significantly ($P < 0.05$) decreased LDL levels (1.14-fold) and increased HDL (1.30-fold) as compared to the diabetic control group, whereas supplementation with commercial oat–wheat cookies increased HDL levels (1.47-fold).

Regarding the atherogenic index, all treatments significantly ($P < 0.05$) decreased this parameter as compared to the diabetic control group (1.38–2.42-fold), and the highest dose of oat–bean cookies (1.2 g kg⁻¹) exerted the greatest effect, presenting similar values to the healthy control group. Similarly, all treatments decreased serum C-reactive protein levels as compared to the diabetic control group (1.23–1.38-fold), and the greatest beneficial effect was observed with the highest dose of oat–bean cookies (1.2 g kg⁻¹).

Effect of oat–bean flour cookies on serum hepatic injury markers in diabetic rats

The diabetic control group presented a significant ($P < 0.05$) increase in the activity of ALT (1.47-fold) as compared to the

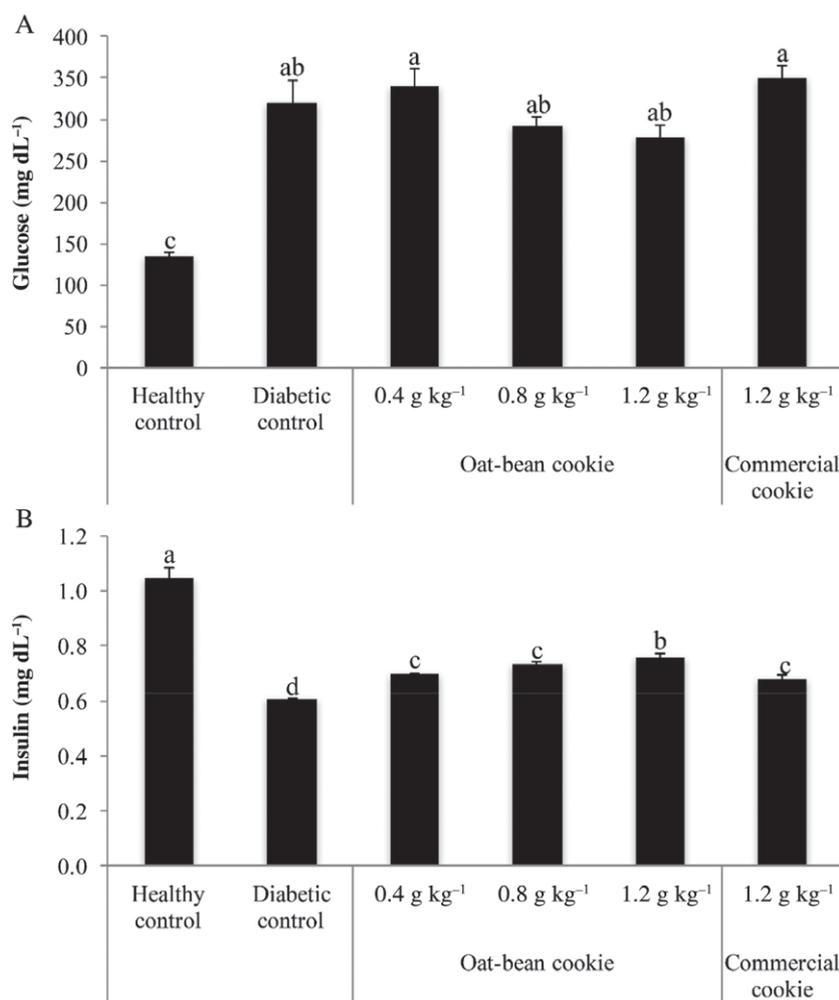


Figure 3. Effect of oat-bean cookies on serum glucose (A) and insulin (B) in high-fat diet and STZ-induced diabetic rats. Data are presented as the mean \pm standard error ($n = 7$). Different letters within each column indicate significant ($P < 0.05$) difference by Tukey's test.

Table 3. Effect of oat-bean flour cookies on serum lipid profile, atherogenic index and C-reactive protein in high-fat diet and STZ-induced diabetic rats

Group	Triglycerides (mg dL ⁻¹)	Cholesterol (mg dL ⁻¹)	LDL (mg dL ⁻¹)	HDL (mg dL ⁻¹)	Atherogenic index (UA)	C-reactive protein (μ g mL ⁻¹)
Healthy control	82.6 \pm 2.6c	36.5 \pm 1.8d	58.7 \pm 1.0d	34.9 \pm 2.1ab	1.1 \pm 0.1d	178.0 \pm 10.3d
Diabetic control	150.3 \pm 5.6a	57.8 \pm 1.6a	90.1 \pm 1.2a	20.3 \pm 0.6d	2.9 \pm 0.1a	428.9 \pm 15.4a
Oat-bean cookie (0.4 g kg ⁻¹)	161.8 \pm 3.5a	53.9 \pm 1.8ab	77.7 \pm 0.7b	26.4 \pm 1.7c	2.1 \pm 0.1b	381.0 \pm 21.1ab
Oat-bean cookie (0.8 g kg ⁻¹)	120.0 \pm 8.4b	47.6 \pm 1.0bc	65.0 \pm 0.4c	30.9 \pm 1.4bc	1.6 \pm 0.1c	330.3 \pm 17.9bc
Oat-bean cookie (1.2 g kg ⁻¹)	108.0 \pm 5.5b	42.5 \pm 1.4 cd	62.1 \pm 0.6 cd	35.4 \pm 0.7ab	1.2 \pm 0.0d	288.1 \pm 15.4c
Commercial oat-wheat cookie (1.2 g kg ⁻¹)	158.4 \pm 5.4a	52.6 \pm 1.8ab	86.4 \pm 1.6a	29.8 \pm 0.6bc	1.8 \pm 0.1bc	312.2 \pm 14.6bc

Data are presented as the mean \pm standard error ($n = 7$). Different letters within each column indicate significant ($P < 0.05$) difference by Tukey's test.

healthy control group (Table 4). All doses of oat-bean cookies slightly decreased ALT activity values as compared to the diabetic control group (1.18–1.22-fold), but no significant ($P < 0.05$) difference was observed.

On the other hand, the diabetic control group presented similar AST activity values to the healthy control group. Nevertheless, all doses of oat-bean cookies significantly ($P < 0.05$) decreased this parameter as compared to the diabetic control group (1.43–1.47-fold), whereas no difference was observed

between the commercial oat-wheat cookie-treated group and the diabetic control group.

DISCUSSION

As shown in Table 1, oat-bean cookies showed a greater content of TDF, SDF and IDF, which, interestingly, were not detected in the commercial oat-wheat cookies. This may suggest that the actual amount and quality of the material used to elaborate the

Table 4. Effect of oat–bean flour cookies on hepatic damage serum markers in high-fat diet and STZ-induced diabetic rats

Group	ALT (U L ⁻¹)	AST (U L ⁻¹)
Healthy control	12.8 ± 0.6c	38.4 ± 2.1a
Diabetic control	18.9 ± 2.1ab	39.7 ± 3.3a
Oat–bean cookie (0.4 g kg ⁻¹)	15.5 ± 0.8b	22.6 ± 1.3c
Oat–bean cookie (0.8 g kg ⁻¹)	15.4 ± 1.1b	21.0 ± 1.1c
Oat–bean cookie (1.2 g kg ⁻¹)	14.8 ± 1.1b	21.1 ± 3.7c
Commercial oat–wheat cookie (1.2 g kg ⁻¹)	22.3 ± 2.3a	34.7 ± 2.9ab

Data are presented as the mean ± standard error ($n = 7$). ALT, alanine transaminase; AST, aspartate transaminase. Different letters within each column indicate significant ($P < 0.05$) difference by Tukey's test.

commercial cookies are not adequate. Oat–bean cookies showed a higher content of stachyose and raffinose, which are commonly found in common beans.

Regarding the phytochemical profile, oat–bean cookies showed a greater content of polyphenols, saponins and phytosterols than commercial oat–wheat cookies, which also demonstrates the positive effect of pinto bean flour addition to the nutraceutical potential of cookies. The commercial cookies used in this study are elaborated with oat and wheat flours; however, several representative polyphenols of these cereals, such as ferulic and sinapic acids, were not detected in these cookies. Furthermore, commercial oat–wheat cookies showed a relatively high amount of catechins, phaseoside I and soysaponins (mainly found in legumes), which may be related to the content of soy derivatives in these cookies.

Several studies have reported the effect of common beans on glycemic response, and thus their consumption is recommended for the prevention and treatment of hyperglycemia and hyperinsulinemia.⁹ This beneficial effect has been widely associated with their high content of several bioactive compounds, such as polyphenols, saponins and phytosterols, as well as galacto-oligosaccharides and dietary fiber, including RS and, particularly, retrograded starch.^{24,25}

Despite all the well-known benefits of common beans, their use as functional ingredients has not been exploited so far. Therefore, we evaluated the effect of cookies elaborated with oat and common bean (Pinto Saltillo cultivar) on diabetes and its complications. In this study, we demonstrated that bean flour may represent a feasible alternative to enrich processed products with phytochemicals, such as polyphenols, phytosterols and saponins, as well as dietary fiber and galacto-oligosaccharides.

The administration of 1.2 g kg⁻¹ oat–bean cookies to HFD and STZ-induced diabetic rats decreased glucose serum levels and increased insulin serum levels. Furthermore, this treatment decreased the hyperglycemic peak in the oral glucose tolerance assay in healthy rats. Therefore, the hypoglycemic effect of oat–bean cookies may be related to an increased insulin production and/or secretion, as well as a decreased glucose intestinal absorption.

The hypoglycemic effect of oat–bean cookies may be related to their dietary fiber content. Both oat and common beans are considered as low glycemic index (GI) foods due to their dietary fiber (soluble and insoluble) and RS content.²⁶ It has been reported that soluble dietary fiber attenuates glucose absorption rate, due to its high viscosity in the small intestine, leading to carbohydrate

entrapment. Moreover, oat β -glucan has been reported to reduce postprandial glucose and insulin response in healthy men.²⁷ Similarly, it has been reported that galacto-oligosaccharides decreased blood glucose in overweight adults.²⁸ Regarding insoluble dietary fiber, it has been reported that its administration in obese rats improved insulin sensitivity.²⁹

Furthermore, the hypoglycemic effect of the highest dose (1.2 g kg⁻¹) of oat–bean cookies may be related to its high content of several phytochemicals. For instance, it has been reported that rutin decreases serum glucose in STZ-induced diabetic rats by modulating the activity of hepatic glycolytic and gluconeogenic enzymes, such as hexokinase, glucose-6-phosphatase (G6Pase) and fructose-1,6-biphosphatase.³⁰ Moreover, the administration of caffeic acid and protocatechuic acid increases glucose uptake in adipocytes by increasing the expression of glucose transporter-4 (*Glut4*),^{31,32} whereas β -sitosterol increases glucose uptake in myotubule cells by promoting the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK), which promotes GLUT4 translocation and expression, leading to decreased serum glucose levels.³³

On the other hand, the supplementation with commercial oat–wheat cookies increased serum glucose levels in diabetic rats, exerting a detrimental effect. Conversely, several phenolic compounds found in high amounts in these cookies have been reported to exert hypoglycemic effects. For instance, chlorogenic acid inhibits hepatic G6Pase activity³⁴ and stimulates glucose transport in skeletal muscle via AMPK activation.³⁵ Furthermore, catechin and epicatechin have been reported to inhibit α -glucosidase activity,³⁶ which is involved in carbohydrate intestinal breakdown. Therefore, the beneficial effects of bioactive compounds of commercial cookies may not counteract the detrimental effect of a high glucose intake, as observed in increased glucose levels after the administration of commercial oat–wheat cookies.

Hyperglycemia and hypoinsulinemia lead to the alteration of lipid metabolism, and thus the development of dyslipidemia, which is considered a risk factor for cardiovascular diseases. Therefore, the hypolipidemic effect of oat–bean cookies was evaluated. The administration of 0.8 and 1.2 g kg⁻¹ oat–bean cookies improved serum lipid profile, as observed in decreased triglycerides, cholesterol and LDL levels, as well as increased HDL levels, presenting a greater beneficial effect than commercial oat–wheat cookies. Furthermore, these treatments decreased atherogenic index and serum C-reactive protein levels, suggesting a cardioprotective potential.

The beneficial effect of the high dose (1.2 g kg⁻¹) of oat–bean cookies may be related to its hypoglycemic effect, since glucose promotes the activity of carbohydrate response element binding protein-1, which promotes the expression of genes involved in fatty acid synthesis, such as fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD) and acetyl-CoA carboxylase (ACC), increasing serum free fatty acid levels and therefore triglyceride concentration.³⁷

The hypolipidemic effect of oat–bean cookies may be related also to their content of dietary fiber and galacto-oligosaccharides, since SDF retards triglyceride and cholesterol intestinal absorption due to its high viscosity.²⁸ SDF and galacto-oligosaccharides have been reported to reduce triglyceride and cholesterol serum levels via the production of short-chain fatty acids, which modulate hepatic lipid metabolism.³⁸ Similarly, IDF has been reported to increase *Foxa2* and *Pgc-1b* expression, which concomitantly promote hepatic fatty acid β -oxidation.²⁹

The hypotriglyceridemic and hypocholesterolemic effects of the oat–bean cookies may be related also to its phytochemical composition. For instance, chlorogenic acid, the major polyphenol of this cookie, reduces the activity of hepatic 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMGCR), and increases the hepatic activity of carnitine palmitoyl transferase (CPT-1), thus decreasing serum cholesterol and triglycerides.³⁹ Moreover, rutin and protocatechuic acid have been reported to decrease the expression of lipogenic enzymes, such as sterol response element binding protein-1 (SREBP-1), FAS, ACC, SCD and HMGCR.^{40,41} Similarly, common bean phytosterols have been reported to decrease hepatic lipogenesis via FAS and SREBP-1c inhibition, and increase fatty acid β -oxidation via CPT-1 activation.¹⁰

Furthermore, phytosterols, such as β -sitosterol and sitosterol- β -3-D-glucoside, as well as soyasaponins decrease cholesterol micellar formation, leading to decreased intestinal absorption.^{42,43} Accordingly, the administration of phytosterol-enriched spread in diabetic pregnant women improved insulin resistance and lipid profile.⁴⁴ Similarly, it has been reported that the consumption of 2 g phytosterols in food products such as margarine, mayonnaise, orange juice, olive oil, low-fat milk and yogurt is associated with significant reductions in LDL levels.⁴⁵

On the other hand, the administration of commercial oat–wheat cookies increased HDL levels in diabetic rats, presenting no effect on other serum lipids. Furthermore, this treatment reduced serum C-reactive protein levels. Accordingly, it has been reported that β -glucan, an important component of oat dietary soluble fiber, increases HDL levels in overweight and hypercholesterolemic individuals.⁴⁶ Regarding the anti-inflammatory effect of the commercial oat–wheat cookies, there are no reports about any biological activity related to inflammation for the major components identified in this cookie.

Diabetes leads to the development of other complications, such as hepatic injury, which is mainly a consequence of hyperglycemia-induced oxidative stress and inflammation. Hyperglycemia increases the production of reactive oxygen and nitrogen species (ROS and RNS), increasing the synthesis of pro-inflammatory cytokines, such as tumoral necrosis factor- α and interleukin-6.²

In liver, ROS/RNS and pro-inflammatory mediators trigger mitochondrial dysfunction of hepatocytes, leading to cell death by apoptosis and necrosis, resulting in the leakage of several enzymes, such as ALT and AST, which are considered markers of hepatic injury.^{47,48} Accordingly, the diabetic control group presented slightly augmented ALT, whereas no alteration was observed in AST, suggesting early hepatic injury. No clear trend was observed with supplementation with oat–bean cookies on hepatic injury since serum ALT was not affected and AST level was decreased.

CONCLUSION

The administration of 1.2 g kg⁻¹ of oat–bean cookies slightly decreased serum glucose levels, which was associated with an increased insulin production and/or secretion, as well as decreased glucose intestinal absorption. Oat–bean cookies (0.8 and 1.2 g kg⁻¹) exerted a greater hypolipidemic effect than commercial oat–wheat cookies (1.2 g kg⁻¹), as observed by decreased serum triglycerides and LDL. Furthermore, supplementation with 1.2 g kg⁻¹ oat–bean cookies exerted the greatest beneficial effect on the atherogenic index and C-reactive protein, suggesting their cardioprotective potential. Regarding hepatic injury, no beneficial

effect was observed with the administration of oat–bean cookies. The beneficial effect of oat–bean cookies was associated with their content of dietary fiber, RS and galacto-oligosaccharides, as well as their high content of chlorogenic acid, rutin, protocatechuic acid, caffeic acid, β -campesterol, β -sitosterol and soyasaponins. These results suggest that pinto bean flour could be used as a functional ingredient for the elaboration of bakery products, such as cookies, with an anti-diabetic effect.

ACKNOWLEDGEMENTS

This work was supported by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campus Zacatecas.

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